Measurement of malaria transmission in Africa; an entomological perspective

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Dedicated to my family

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Summary

Introduction

Understanding the relationship between malaria transmission intensity and malaria related morbidity is essential for effective malaria control. There is renewed interest on eliminating malaria worldwide that has been followed up with rapid and wide scale deployment of different malaria control interventions. Monitoring the impact of these interventions on malaria transmission dynamics should ideally be done in parallel with these efforts. This can be achieved by tracking levels of key parameters in malaria transmission dynamics, such as parasite rates in mosquitoes and humans, exposure of humans to mosquitoes, sensitivity of mosquitoes to interventions, sensitivity of malaria parasites to chemotherapy. These parameters are likely to vary from one area to another depending on the nature of local malaria transmission epidemiology. Entomological inoculation rate (EIR) is a direct measure of malaria transmission, but it is rarely measured across endemic areas as a result of the being laborious and liable to vary greatly from area to another and across seasons. Here two study platforms, the Malaria Transmission Intensity and Mortality Burden across Africa (MTIMBA) and Malaria Transmission Consortium (MTC), span different sites and provided an opportunity to explore historical as well as more recent dynamics of mosquito mediated malaria transmission. Study sites reported in this thesis are found in Burkina Faso, Ghana, Kenya, Mozambique, Tanzania and Zambia. The two studies were implemented at different time periods, the MTIMBA project covers the period between 2001-04, while the data from the MTC project was from 2009-10. These periods conveniently offer an opportunity to study the different mosquito populations prior and after large scale rollout of vector control across malaria endemic areas.

Results

Two study sites from Tanzania, one site called Rufiji having artemisin based combination therapy (ACTs) and the other Kilombero-Ulanga as the control where sulphadoxine-pyrimethamine (SP) was used for treatment of malaria infection across all ages. Artemisins are known to have an effect of killing gametocytes, the transmissible stage of the malaria parasite from humans to mosquitoes. In this study artemisin (AS) was combined with suphadoxine pyrimethamine (SP). Based on measurement of the rate of oocysts in mosquito population, it was possible to determine the impact of using ACTs on reducing the reservoir of malaria parasites in human population in an area of intense malaria transmission. The introduction of AS+SP in Rufiji was associated with increased oocyst prevalence (OR [95%CI] = 3.9 [2.9-

5.3], p < 0.001), but had no consistent effect on sporozoite prevalence (OR [95%CI] = 0.9 [0.7-1.2], p = 0.5). These outcomes may be a result of large variations in emergence rates and survival of mosquitoes in this region, and cannot be accounted for by the change in treatment of malaria. In an area where humans are more likely to have chronically asymptomatic infections, malaria case management should be supplemented with other interventions that can drastically reduce the level of malaria transmission intensity for added benefits of suppression of human infectiousness to mosquitoes.

Another mosquito based parameter necessary to gauge the amount of human exposure that occurs indoors, and therefore can be prevented by indoor based vector control was estimated across six mosquito populations. This estimate, termed as the proportion of human exposure to both *Anopheles gambiae* sensu lato and *An. funestus* s.l. that occurs indoors (π_i), is used as a measure of the upper limit for the personal protection that indoor vector control measures can provide. Across these mosquito populations, neither *An. gambiae* s.l. nor *An. funestus* s.l. strongly preferred feeding indoors (P_i = 0.40 – 0.63 and 0.22 – 0.69, respectively) but they overwhelmingly preferred feeding at times when most humans were indoors (P_{fl} = 0.78 – 1.00 and 0.86 – 1.00, respectively). Since the majority of humans spend most of the time indoors at night then the majority of human exposure to *Anopheles* bites occurred indoors (π_i^B = 0.79 – 0.97). These results are in favour of the ongoing efforts of increasing coverage of indoor based personal protection measures such as insecticide treated nets (ITNs) and insectide residual spraying (IRS) across Africa. Such estimates also provide measurable parameters that can be used to longitudinally monitor the levels of protection that ITNs and IRS can reasonably offer against a given vector population. Also caution is raised on the existence of outdoor exposure that has to be targeted by complementary measurers for absolute reduction of malaria transmission.

Estimation of the rate of contact between mosquitoes and humans is essential in measuring the level of malaria transmission intensity. This parameter is liable to vary from one person to another as well as from one population to another, but yet estimation of this parameter has to be standardized. Human landing catch (HLC) is thus far the gold standard for measuring human biting rates. Centers for disease control light traps (LT) have been used widely in malaria endemic setting as an alternative tool to HLC in estimating human biting rate (HBR). Sampling efficiency of LT against HLC has been reported to be variable in different independent studies. These differences might be as a result of different methodologies that are applied when analysing these data. Here regression models were applied to

determine the site specific as well as the overall LT sampling efficiency for the two major malaria vectors across Africa, *An. gambiae* sensu lato and *An. funestus* sensu lato. Generally, LT were able to collect more mosquitoes than HLC, though the ratio of LT: HLC varied between sites and mosquito density. Across sites LT had an overall sampling efficiency of $\tilde{\alpha}_t$ =1.07 [0.76-1.51] in sampling *An. gambiae* s.l. and $\tilde{\alpha}_t$ =1.78 [0.90-3.44] in sampling *An. funestus* s.l.. There was variation in sampling efficiency of LT across mosquito densities and only in a few locations did LT sample proportionally to HLC. These observed inconsistencies may be a result of differences in implementation of the HLC and LT calibration exercise, necessitating the need for local calibration of LT against HLC for each location and across seasons.

Conclusion

It is necessary to monitor vector populations as part of epidemiological studies of malaria transmission dynamics. The existence of different local malaria transmission dynamics, make malaria control difficult. Selection of key parameters such as those presented here and establishment of standardized study procedures can aid in providing a means of monitoring mosquito populations and their response to ongoing interventions. Such efforts require long-term commitment as well as selection of some sentinel sites upon which longitudinal measurements of for example the proportion of human exposure occurring both indoors and outdoors, levels of responsiveness to ongoing insecticide based vector control interventions can be regularly measured. This together with centralized data storage and access, then real-time status of mosquito populations can be made available for proper planning and implementation of malaria control interventions.

1. General introduction

1.1 Malaria: burden and distribution

Malaria, a disease caused by protozoans of the genus *Plasmodium* and transmitted by female mosquitoes of the genus *Anopheles,* is among the most serious health problems facing the developing world. The risk of malaria transmission is highest in Sub Saharan Africa, Asia and the Americas, but among these regions, Sub-Saharan Africa has the highest burden (WHO 2012). This is mostly due to the predominance of the most efficient vectors-parasite combination between the vectors *Anopheles gambiae* and *Anopheles funestus* with the *Plasmodium falciparum* parasite, accompanied by favourable environmental conditions of temperature, rainfall and humidity (Gillies and DeMeillon 1968; Hay, Guerra et al. 2009). However, the condition of malaria in this region is made worse by poor health systems that fail to reach the neediest and most malaria affected rural communities. Lack of a balance between soaring human population growth and improvement of public health systems may account for this failure.

Recent increased efforts made by global alliances on controlling malaria, have led to a 26% global reduction in malaria specific mortality rates, while in Africa alone the reduction was about 33% between the years 2000 and 2010 (WHO 2011). Furthermore, between the year 2001 and 2010, 274 million less cases as well as 1.1 million less death were averted globally based on the baseline incidence and mortality estimates of the year 2000 (WHO 2012). These lives were saved as a result of increased access to funding for malaria control, that led to an up-scale of protective interventions such a insecticide treated bednets (ITNs) and long lasting insecticide treated nets (LLINs) from 3% to 53% of households owning at least a single net, use of insecticide residual spraying (IRS) has increased from 5% in 2005 to 11% in 2010, improved diagnosis by use of rapid diagnostic tests (RDTs) together with treatment of infected persons by efficacious artemisin based combination therapy (WHO 2012; Alonso and Tanner 2013). Nevertheless globally, in the year 2010 alone there was still an estimated 216 million malaria cases (uncertainty range 149 million to 274 million) that resulted in about 655,000 (uncertainty

range 537 000 to 907 000) deaths (WHO 2011). The bulk of this incidence and mortality is on the most immunologically naïve of the population, that is children under-five year old and pregnant women.

Morbidity and mortality associated with malaria is high, to the extent of being associated with 1.3 % reduced economic growth in malarious compared to non-malarious countries (Gallup and Sachs 2001). The economic cost of malaria at a household level is highest among the poor such that malaria has been found to be strongly associated with poverty (Sachs and Malaney 2002). Reports show that countries with the highest proportion of their citizens living in poverty, that is on less than US $1.25 per person per day, harbour the highest burden of malaria in the world (WHO 2012). The poorest quintile among these human populations bears the highest burden of malaria parasite prevalence, as result of increased chance of exposure to infectious mosquitoes, due to poor living conditions and lack of access appropriate treatment. Lowering the burden of malaria among African countries may pave the way for economic growth, at the household level and at large by reallocation of government expenditure to improve other sectors of the health system.

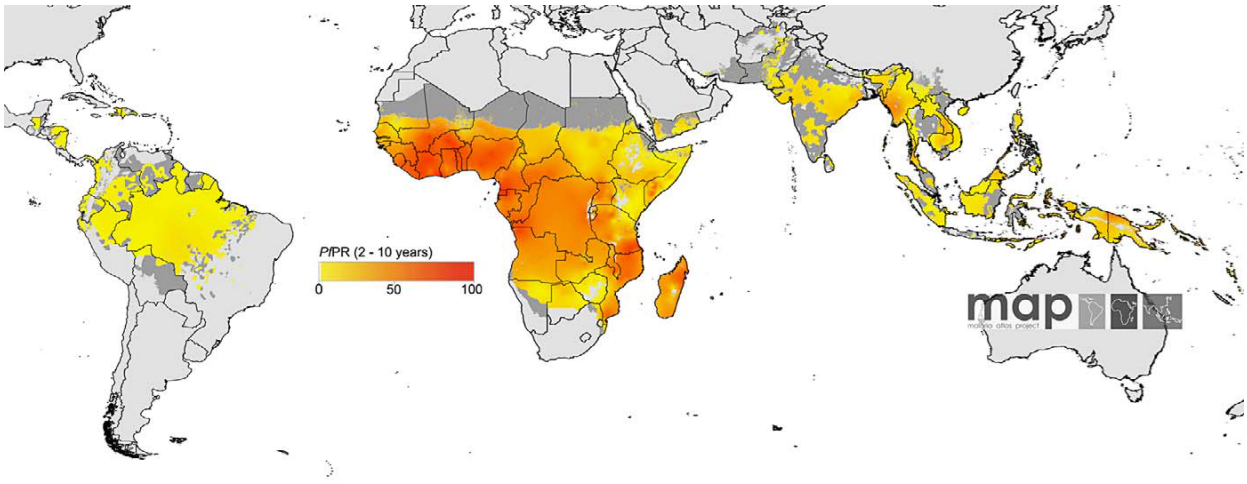


Figure 1 The spatial distribution of *P. falciparum* malaria across the world (Hay, Guerra et al. 2009)

1.2 Malaria transmission

1.2.1 Major vectors of malaria in sub Saharan Africa

Malaria parasites are transmitted by female mosquitoes, belonging to the phylum arthropoda, class insecta, order diptera, family culicidae and anopheline genera. The genus *Anopheles* is composed of more that 400 species, among these approximately 60 species are able to transmit malaria and therefore are of major medical importance (Bruce-Chwatt 1985). Female anophelines are haematophagous that is they feed on blood of warm blooded animals including humans and it is through this process that they serve as vectors of malaria parasites.

Anophelines are found in different areas of the world, co-existing in different species combinations and frequencies as determined by environmental conditions and mosquito adaptations (Gillies 1988)(Table 1). *Anopheles gambiae* sensu lato is a complex of seven cryptic species while *Anopheles funestus* sensu lato forms a group of about eleven subspecies that are morphologically similar as adults but can be easily distinguished by using molecular methods (Gillies and DeMeillon 1968; Coluzzi, Sabatini et al. 2002; Choi, Koekemoer et al. 2012). Members of these complexes vary in their ability to transmit malaria mainly based on their host preference between humans and other animals. In nature these mosquitoes have been reported to co-exist with one another in different combinations as summarized in Table 1 (Gillies and Coetzee 1987). In addition to these major vectors, *Anopheles nili* and *Anopheles moucheti* mosquitoes have been associated with malaria transmission in Sub-Saharan and central Africa respectively (Gillies and DeMeillon 1968).

Table 1 Major malaria transmitting anophelines across Sub-Saharan Africa

Mosquito taxon	Genetic polymorphism\species	Reported bionomics and behaviour	Distribution
An. funestus group	*An. funestus* s.s.	▪ Anthropophagic and endophilic and endophagic. ▪ Prefers to breed on more or less permanent water bodies preferably shaded by vegetation such as rice fields.	Sub-Saharan Africa
	An. rivulorum	▪ Zoophilic, exophilic and exophagic.	
	An. confusus	▪ Zoophilic, exophilic and exophagic.	
	An. leesoni	▪ Zoophilic, exophilic and exophagic.	
	An. brucei	▪ Zoophilic, exophilic and exophagic.	
	An. parensis	▪ Zoophilic, exophilic and exophagic.	
	An. aruni	▪ Zoophilic, exophilic and exophagic.	
	An. vaneedeni	▪ Zoophilic, exophilic and exophagic.	
An. gambiae complex	*An. gambiae* s.s.	▪ Breed in temporary and permanent stagnant water usually associated with human disturbances. ▪ Anthropophilic and endophilic. ▪ Sympatric with *An. arabiensis* as larvae and adults	▪ Sub-Saharan Africa
	An. arabiensis	▪ Turbid water, lacking aquatic vegetation or surface film. Breed near cattle. Prefer both humans and cattle equally depending on their availability. ▪ Adults bite humans indoors and outdoors but also cattle, after feeding rests both indoors and outdoors.	▪ Sub-Saharan Africa, tends to occur in drier areas than *An. gambiae*
	An. melas	▪ Breeds in salt brackish water ▪ Anthropophilic and equally both endophagic and exophagic.	▪ West coast of Africa. ▪ Not sympatric as larvae with any co-specific, but as adults may be sympatric with *An. gambiae* and *An.arabiensis*
	An. merus	▪ Breeds in salt brackish water ▪ Zoophilic and exophagic	▪ East and south coast of Africa. ▪ Not sympatric as larvae with any co-specific, but as adults may be sympatric with *An. gambiae* and *An. arabiensis*
	An. quadrianulatus	▪ Feeds largely on cattle, not regarded as malaria vector.	▪ South East Africa and Ethiopia. ▪ Sympatric with *An. arabiensis* only
	An. bwambae	▪ Breeds in mineral springs, locally it can transmit malaria.	▪ Semliki forest in Uganda. ▪ Sympatric with *An. gambiae* as adults
An. nili group	*An. nili s.s*	▪ Breeds on edges of large rivers and streams. ▪ Efficient vector with sporozoite rate of up to 3%. ▪ Anthropophilic, both endophagic and exophagic.	▪ Throughout Africa except in the Sahel region
	An. somalicus	▪ Zoophilic	
	An. carnevalei *An. ovengensis*		
An. moucheti group	*An moucheti moucheti*	▪ A forest species, breeds on the edges of slow moving streams, rivers, pools or ponds, where the canopy is broken or where there is penetration of light. ▪ Highly anthropophilic and an efficient vector sporozoite rate up to 4%.	▪ Forest mosquitoes present in Equatorial Africa from Guinea to Uganda and South Sudan
	An. moucheti nigeriensis		
	An. bervoetsi		▪ Congo only

Life cycle of mosquitoes

Mosquitoes have four life stages which are eggs, larvae, pupe and adults (Figure 2). Eggs are laid singly by female anophelines on fresh water except those of *An. merus* and *An. melas* that may be laid on salty water (Table 1). The size and conditions of a breeding habitat is dependent on the species of mosquitoes, ranging from open sunlit bodies in *An. gambiae* to shaded banks of rivers in *An. funestus* (Gillies and Coetzee 1987). Depending on the ambient temperature, eggs may hatch and release larvae in 2 – 3 days in the tropics where ambient temperature is high and 2 - 3 weeks in colder climates.

Anopheline larvae feed by filtering planktons and organic debris dispensed in water, they tend to position their bodies parallel to the surface of water and breathe through spiracles located on the abdomen. Larvae have four aquatic instars that vary in their duration depending on the ambient temperature. In the tropics where it is much warmer, maturation of mosquitoes from the aquatic stages to adults can occur within a week as opposed to longer periods in cooler climates. The fourth larvae stage transforms into a comma shaped non feeding pupae that often rise to the water surface to breathe through a pair of respiratory trumpets. Aquatic mosquito stages are limited by predation and food availability (Koenraadt, Majambere et al. 2004), the latter may affect both the quality and quantity of emerging adult mosquitoes. Nutritional deprivation as larvae may results into small adult mosquitoes, with reduced competitive advantage while seeking for a mate (Yuval, Wekesa et al. 1993) and while host seeking (Takken 1998), as well as have lowered immune response to *P. falciparum* infection (Suwanchaichinda 1998) and reduced survival (Ameneshewa 2008).

The pupae moult into adult mosquitoes, usually in the evening, coinciding well with the time for nuptial flight that occurs mostly at dusk and occasionally at dawn. Mating can occur on the first day of emergence in female anophelines but can be delayed for a 24 hours in males, to allow the maturation of the sexual organs and inversion of the terminalia 180^0, the latter is associated with proper orientation during mating (Charlwood and Jones 1979; Verhoek and Takken 1994; Howell and Knols 2009). A nuptial flight is ritualised by formation of a swarm of

male mosquitoes that aggregate above a marker (Charlwood and Jones 1979; Marchand 1984; Charlwood, Pinto et al. 2002; Charlwood, Thompson et al. 2003). Female mosquitoes fly into or close to a swarm, and are recognized by their lower wing beat frequency (Clements 1963; Charlwood and Jones 1979; Charlwood and Jones 1980). A successful courtship results in mating and storage of spermatozoa into a spermatheca. Female mosquitoes usually mate only once, and therefore use spermatozoa stored in the spermatheca for subsequent fertilization of her eggs. Monogamy in female anophelines, is induced by a proteinaceous mating plug that is inserted into the genital chamber by male mosquitoes in the process of mating (Chambers 2001). The plug prevents successful inseminations by other males upon further mating attempts.

Male anophelines feed exclusively on plant nectar as an energy source, while females need plant sugars for energy but also proteins contained in blood of some animals for proper development of their eggs. Host seeking may occur prior or after mating depending on the mosquito's physiological status and age (Jones and Gubbins 1978). Occasionally female mosquitoes mate before host-seeking, but more often mosquitoes host-seek after mating (Gillies and Coetzee 1987; Charlwood, Pinto et al. 2003).

Adult mosquitoes are ectothermic, that is they are dependent on the environmental temperature for regulation of their metabolic activities. The duration of their life is dependant on ambient the temperature, humidity levels, resource availability and coverage of those resources with vector control interventions. Free-living wild anophelines can survive for an average of one to two weeks in the tropics, though there have been records of mosquitoes surviving up to more than six weeks in the wild (Gillies and Wilkes 1963) and up to four weeks when reared in insectaries. These variations are a proof of the dependency of the environment on survival of mosquitoes.

Mosquito population dynamics can be influenced by rainfall, temperature and altitude. Rain can create breeding habitats for aquatic stages of mosquitoes as well as regulate the moisture content of the air, which is the humidity. Drought as result of less rain and therefore low humidity reduces the number and quality of breeding habitats for aquatic stages of the malaria

parasite, as well as reduce survivorship of adult mosquitoes due to desiccation. Increased rainfall can in some situations increase the stability of breeding sites or alternatively wash out other habitatxs. Heavy rainfall may affect *An. gambiae* populations more since they are more likely to breed in small collections of water left behind by rain as opposed to *An. funestus* that can breed on the edges of large permanent bodies of water. Extremes of temperature have negative impacts on transmission of malaria, limiting the geographical boundaries of malaria transmission worldwide. Ambient temperature is low at high altitudes restricting the development and survival of *Anopheles* therefore making malaria essentially uncommon in highlands

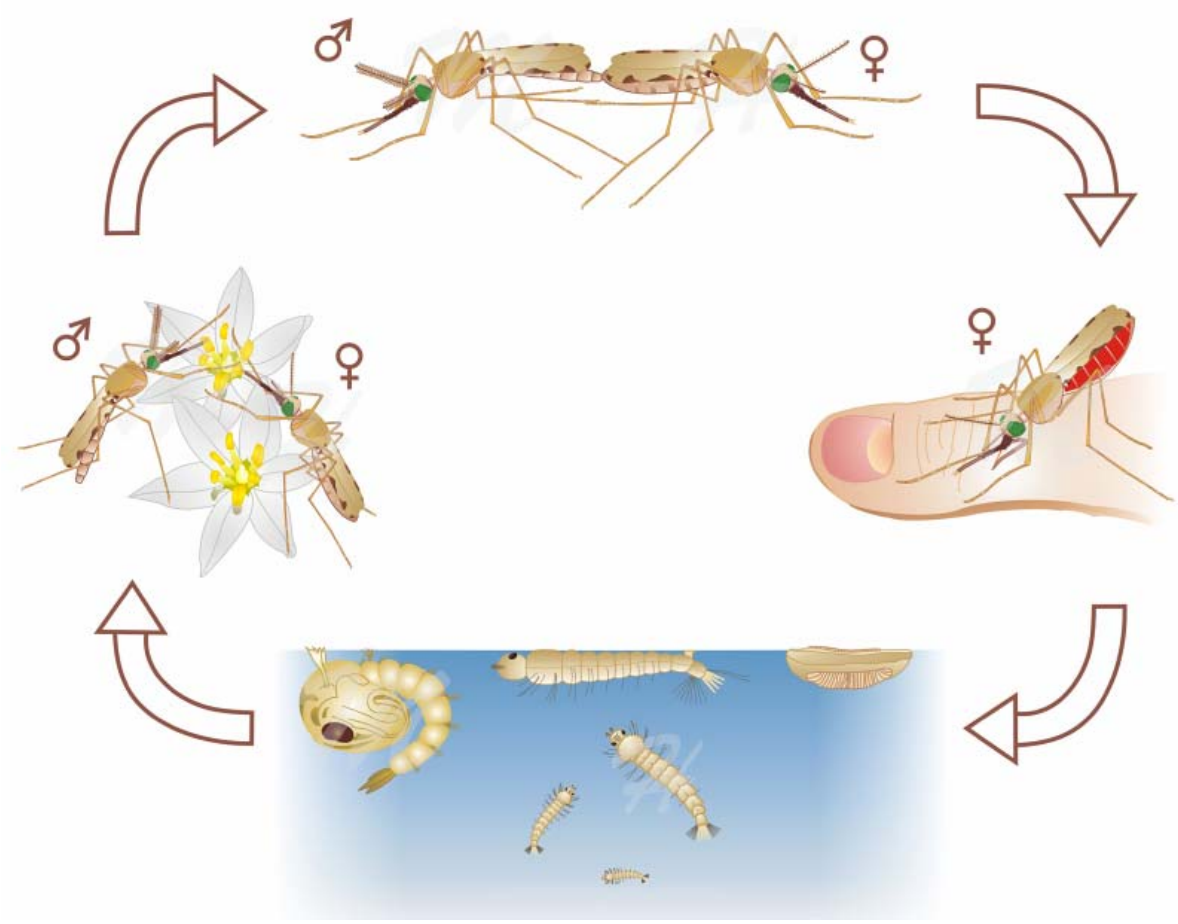


Figure 2 Developmental stages of mosquitoes from eggs to adult (Source: **http://www.biographix.cz/portfolio/schemes-models/life-cycle-of-the-mosquito-anopheles-gambiae/**)

1.2.2 The parasite

Malaria is caused by protozoans of the phylum apicomplexa, class aconoidasida, order haemosporida, family plasmodiidae and genus plasmodium. Five species belonging to this genus can to lead to human malaria. The species are *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi.* They are heterosexual, with sexual reproduction occurring in mosquitoes and asexual reproduction in humans. Among these plasmodia, *P. falciparum* and *P. vivax* have the highest prevalence, the former is associated with the most severe form of malaria and accounts for most of malaria related morbidity and mortality in sub-Saharan Africa. *P. vivax* has much broader distribution globally even in temperate areas but causes a substantial burden in central Asia and south America (Mendis, Sina et al. 2001). *P. vivax* has a much wider range of temperature that it can persist and multiply while inside the mosquito therefore allowing it to have a wider distribution. Development of *P. falciparum* is optimal between 16 ^{0}C and 19 ^{0}C while *P. vivax* can develop in temperature as low as 14.5 ^{0}C and 15^{0}C (Gage, Burkot et al. 2008). However, the prevalence of the red blood cell disorder known as Duffy blood group antigen deficiency, which causes refractoriness of *P. vivax* infection, has also led to low levels of *P. vivax* prevalence in some parts of Africa. *P. ovale* and *P. malariae* account for a small fraction of malaria infections that occur in Africa. *P. knowlesi* is more common in forested areas of South East Asia, it is a zoonotic causing malaria in macaques a type of monkey belonging to the genus *Macaca* (Lee, Divis et al. 2011).

1.2.3. Malaria transmission cycle

Malaria in general is transmitted to humans by a bite of female anophelines (Figure 3). Malaria parasites ingested in a blood meal are digested except for gametocytes, the sexual form and transmissible stage of the malaria parasite. Mosquitoes are the definitive hosts of *Plasmodium* parasites, therefore sexual reproduction occurs exclusively inside the mosquito. Formation of gametocytes into male microgametocytes and female macrogametocytes, occurs inside the mosquito's midgut upon being triggered by a drop in temperature, a reduction in the

concentration of dissolved carbon dioxide and an increase in pH inside the mosquito's body (Beier 1998).

The nucleus of the microgametes undergoes three rounds of replication resulting into several nuclei that bear flagella. These nuclei emerge from the body of microgametocyte, and as a result of the beating of the flagella the process is termed as exflagellation (Figure 3). While microgametocytes undergo exflagellation, macrogametes mature. Released microgametocytes are highly mobile, they seek and fuse with mature macrogametocytes and result into a diploid zygote that eventually elongates into an ookinate within 12-24 hours. Ookinates avoid being digested in the gut by traversing through the peritrophic membrane, the midgut epithelium and attach to the outer wall of the stomach where they develop into spherical oocysts. Oocysts undergo asexual reproduction called sporogony, which results into production of numerous motile haploid sporozoites that are released into the haemolymph. Sporozoites are able to recognize the salivary glands, traverse the salivary gland epithelia cells and lie within the lumen ready to be expelled when a mosquito takes a blood meal. Sporozoites in the salivary glands remain alive and infective for the remainder of the mosquito's life (Beier 1998). The duration of development of the parasite inside the mosquito, the extrinsic incubation period ranges from 10-28 days and is highly dependant on temperature and is specific for a vector-parasite combination (WHO 1975). Sporogony is time limiting process of the duration of the extrinsic incubation period, as a result of its dependency on ambient temperature, at 28^0C sporogony of *P. falciparum* takes about 9-10 days while that of *P.vivax* takes about 8-10 days, below 18^0C the time needed for development of *P. falciparum* becomes longer than the average lifespan of mosquitoes (WHO 1975).

Sporozoites are injected with saliva when a mosquito feeds on humans, the injected sporozoites enter into the circulatory system and migrate to the liver cells, the hepatocytes, within the first hour of infection (Figure 3). Here they develop into pre-erythrocytic schizonts, that under go schizogony, a type of asexual replication where the parasite undergoes nuclear division that result into production of several progeny called merozoites. In *P. vivax* and *P. ovale* some of the sporozoites skip schizogony and develop into dormant liver stage called

hypnozoites. Hypnozoites may undergo schizogony days, months or even years after an initial attack leading to a relapse of malaria, this strategy is essential to the survival of *P. vivax* (Verhave 2013). Released merozoites infect erythrocytes and develop into trophozites that later on undergo erythrocytic schzogony to release more merozoites that will carry infection to other red blood cells. The rapture of red blood cells leads to release of toxins and parasite antigens that account for the intermittent fevers that are associated with malaria. As an alternative to schizogony some of the released merozoties will differentiate into gametocytes, a stage of the parasite that is transmissible to mosquitoes.

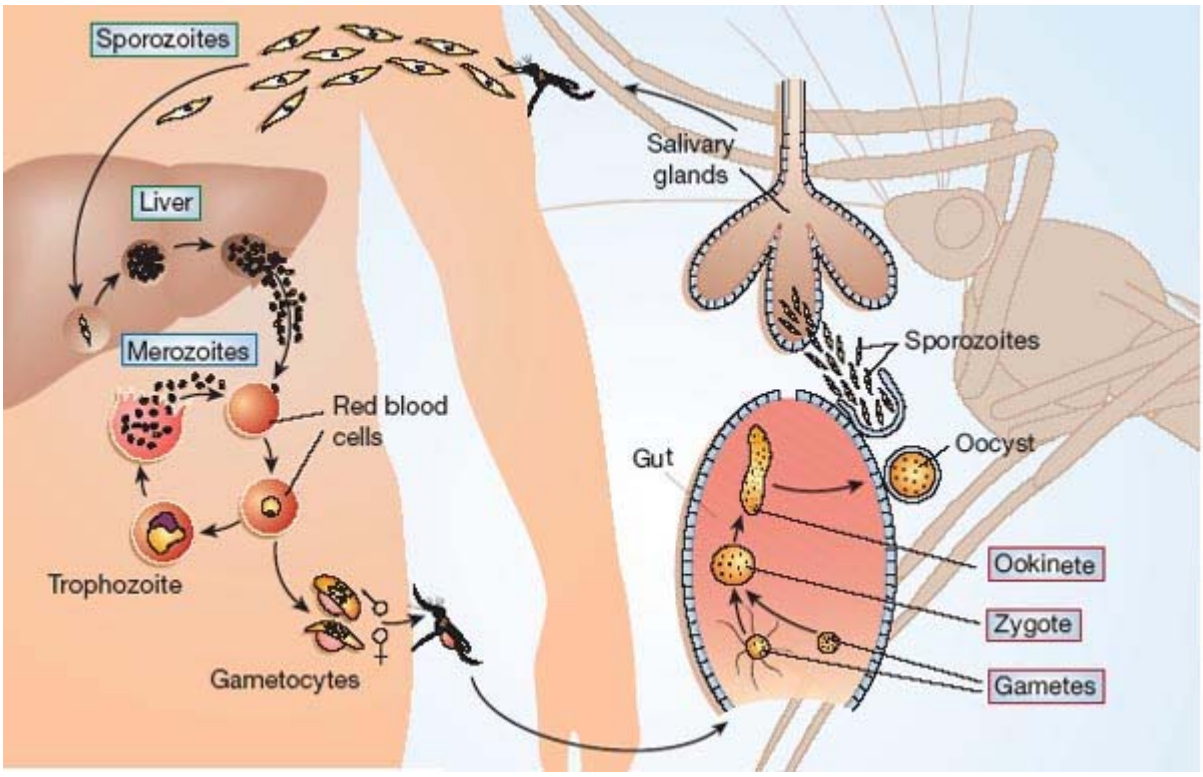


Figure 3 Malaria transmisssion cycle (source :(Ménard 2005))

1.3 Determinants of malaria transmission

The distribution of malaria infections is dependent on factors associated with interactions between humans as hosts, mosquitoes as vectors and *Plasmodium* as a parasite. These interactions are described hereunder.

1.3.1 Human as hosts of malaria infection

Immunity

The severity of *Plasmodium* infection in humans is dependant on the cumulative effects of previous exposure to malaria parasites. Therefore humans living in malarious are more likely to acquire immunity against severe malaria as they get older, this fraction of human population can serve as a cryptic reservoir of malaria parasites at low density but nevertheless infectious to mosquitoes. This accounts for the observed higher burden and severity of malaria infection among children under the age of 5 years as well as immune compromised fractions of the human population such as pregnant women. Infants in malaria endemic settings are protected from severe form of malaria as a result of the acquired post-natal immunity that wanes off depending on the intensity of transmission (Doolan, Dobaño et al. 2009).

Naturally acquired immunity to malaria does not prevent further infection but reduces the severity of the disease in adults (Doolan, Dobaño et al. 2009). However, immunity to malaria infection can be lost with time, following periods of non-exposure, increasing the chances for severe consequences of malaria infection among returning migrants as well among communities where malaria transmission resumes after it had been previously controlled to low levels (Ghani, Sutherland et al. 2009).

Innate resistance towards malaria has been associated with increased prevalence of certain genetic conditions such as sickle cell anemia, duffy negativity, thelassemia and glucose-6-phosphate dehydrogenase deficiency. These genetic conditions limit infected red blood cells from allowing the parasite to reach full maturity, thus terminating infection (Langhorne, Ndungu et al. 2008). Consequently these traits have been selectively favored in malaria endemic human populations as result of the protective effect that they offer against malaria infection.

Behaviour

To a large extent the success of malaria control is highly dependent on human behaviour towards acceptance, use and sustainance of interventions. When faced with limited options of interventions to upscale, choosing an intervention that can be well accepted and integrated into the community may yield higher estimates of protective efficacy against malaria. For example community education through promotional activities has been associated with increased use of bed nets across Africa (Schellenberg, Abdulla et al. 1999).

Humans can increase risk of exposure to malaria transmitting mosquitoes as they engage in their economic activities such as deforestation, clearing land for farming by opening up and creating habitats for mosquitoes to breed. Other mosquito breeding habitats results from pits left following construction, empty containers and tyres that can collect water. Construction of houses that are not proofed for mosquito entry and as a result they offer resting sites for mosquitoes, increasing risk of infection to its inhabitants.

Some occupations keeps humans outdoors at night e.g. security personnel, but it is also common in some parts of the tropics for people to rest outdoors in the evening, especially on moonlit nights in areas where there is no electricity and even sleep outdoors during the hottest period of the year. Such behaviour increases the risk of exposure to potentially infectious mosquitoes and encourages outdoor feeding among the mosquito population (Braimah, Drakely et al. 2005; Yohannes, Haile et al. 2005; Reddy, Overgaard et al. 2011; Russell, Govella et al. 2011; Kawada, Dida et al. 2012; Stevenson, Laurent et al. 2012; Yohanne and Boelee 2012). This fraction of human population can sustain an outdoor biting mosquito population especially in areas where the indoor human population is well covered by exposure reducing malaria interventions.

Protective human behaviour includes planting and sometimes burning of indigenous plants that have a repellent effect towards mosquitoes (Maia and Moore 2011; Ogoma, Moore et al. 2012). Keeping of domestic animals close to homesteads may reduce exposure by offering alternative host choice especially for anophelines that are flexible in their host choice between humans and cattle, e.g. *An. arabiensis* (Tirados, Gibson et al. 2011). On the contrary, animals

may draw more mosquitoes and therefore increase the risk of exposure, either scenario is possible depending on the type of *Anopheles* that predominates in a given setting.

Host attractive factors

In locating a host, mosquitoes use a number of different cues including olfactory cues. Individuals vary in the type of body odour that they produce as a result of the quantity rather than the variety of bacteria that are found on their skin (Verhulst, Qiu et al. 2011). These bacteria are responsible for the producing distinct human body odour, a type of kairomone (Verhulst, Qiu et al. 2011). Human body size may influence the visual cues received by host seeking mosquitoes, adults get more mosquito bites in comparison to children, most likely as a result of their bigger body size or as result of producing more body odour (Takken and Verhulst 2012). Carbon dioxide emitted by vertebrates serves a general cue to mosquitoes by signalling the presence of a host, but host specific cues such as lactic acid, released from the skin as a by product of the excretory process, are necessary for signalling the presence of a specific host to mosquitoes (Takken and Verhulst 2012). Human body heat creates a temperature gradient with the environment that aids the dispersal of these host cues.

Human population distribution: The number of humans in relation to other vertebrates such as cattle can influence the host preference among some mosquito species that are opportunistic in their host choice. Though it may not always be the case, but it is more likely for populations of *An. arabiensis* and *An. rivolurum* to shift biting preference to cattle in the absence of humans, and resort to feeding on humans once they become available (White 1974).

1.3.2 The parasite

The success of a parasite depends on its ability to infect and maintain the infection at levels which are not lethal to its host, so as to maximize the duration that it can persist and maintain transmission. *P. falciparum* can cause infected erythrocytes to keep changing the surface proteins molecules as a mechanism of avoiding being recognized by human immune system, in

a phenomenon known as antigenic variation (Scherf, Lopez-Rubio et al. 2008). *P. vivax* and *P. ovale* produce hynozoites, a dormant stage of the parasite that evades the immune system and chemotherapy but can re-initiate infection at a later time (Mueller, Galinski et al. 2009). Malaria parasites can influence the biting behaviour of mosquitoes, this has been seen in semi field experiments where humans harboring gametocytes were found to be more attractive to mosquitoes than uninfected humans and those with the non transmissible sexual stage of the malaria parasite (Lacroix, Mukabana et al. 2005).

A field study of *An. gambiae* and *P. falciparum* demonstrated that mosquitoes harboring sporozoites, the transmissible stage of *Plasmodium* from mosquitoes to humans, were more likely to be more fully fed, as opposed to being part fed, as well as have blood meals originating from multiple hosts (Koella, Sørensen et al. 1998)

1.3.3 The vector

For a mosquito to transmit malaria, it must survive the whole duration of sporogonic development of the malaria parasite. The duration ranges from 12-23 days (WHO 1975; Koella 1999) and is mostly influenced by the ambient temperature. An efficient vector must have a high affinity for human blood as well as an optimal feeding strategy, when humans are available either indoors or outdoors. Apart from human feeding propensity, its capacity as a vector is mostly dependant on its ability to survive long enough for the parasite to be infective rather than on the size of its population (Dye 1986).

The success of *An. gambiae* s.s. and *An. funestus* s.s. as efficient vectors of malaria in Africa is also partly due to their ability to evolve their life history around humans and their settlements. This includes preference of host seeking indoors at night when humans are asleep, generally referred to as endophagy. Endophagy has evolved together with preference of mosquitoes to rest indoors, also known as endophily. Contrary to these some sub species of these vector complexes have shown preference of biting outdoor, exophagy while preferring to rest outdoors is referred to as exophily (Table 1). In addition *An. gambiae* s.s. and *An. funestus* s.s.

have acquired a high affinity for human blood as opposed to other animals, thus they are referred to as strict anthropophiles.

The density of mosquitoes is influenced by rainfall and temperature therefore they are more abundant at low altitudes in the tropics making malaria and essentially uncommon in temperate areas, including highlands. This is partly due to temperature dependence development of aquatic stages of mosquitoes (Paaijmans, Blanford et al. 2012). Low temperature results in delayed development and increased mortality of larvae, insectary reared larvae of *An. gambiae* have been observed to stop developing at about 16^0C and to start to die at 14^0C. In adult mosquitoes increase in ambient temperature leads to a faster rate of digestion of a blood meal, which can result into increased host seeking, and therefore increased malaria transmission efficiency.

1.4. Quantification of the level of malaria transmission

Prevalence of human malaria can be determined in cross-sectional surveys by deploying diagnostic tools such as microscopy and rapid diagnostic tests (RDTs). While in mosquitoes, sporozoites prevalence can be obtained by ELISA, to give an estimate of the potential infectious mosquito reservoir.

Overtime, the epidemiology of malaria transmission of a given place can be characterized based on the incidence rates of human infection. Endemic transmission is when there are always measurable cases of natural human malaria transmission, as opposed to epidemic transmission that is associated with outbreaks of infection.

Endemic malaria can be classified into different levels based on parasite rates in humans and the entomological inoculation rate (EIR) (Table 2). EIR is expressed as $EIR = MaS$ where Ma is man biting rate for given time and S is the proportion of sporozoites positive mosquitoes for a given species of malaria vector (Beier, Killeen et al. 1999; Shaukat, Breman et al. 2010). Characterizations of malaria transmission intensity are useful in decision making on allocation

of interventions, and in predicting the impact that interventions may have on transmission of malaria.

Table 2 Classification of malaria transmission intensity (WHO 1951)

Parasite prevalence	EIR	Endemicity	Definition
1-10%	< 0.25	Hypoendemic	Little transmission
11-50%	0.25-10	Mesoendemic	Variable transmission intensity depending on local situation
51-75%	11-140	Hyperendemic	Intense seasonal transmission
>75%	>140	Holoendemic	High perennial transmission

EIR entomological inoculation rate

1.5 Malaria control

The current situation

Toward the end of the 1990 there has been renewed interest among the global malaria community towards reducing the global burden of malaria. Previous similar attempts were carried out in 1955-1969 by the Global Malaria Eradication Programme under the World Health Organization (WHO). These campaigns successfully managed to eradicate malaria in developed countries of Americas and Europe, excluding Africa (Snow, Amratia et al. 2012). Africa south of the Sahara malaria transmission was mostly intense and prolonged by long transmission seasons, this region was also faced with poor infrastructure and weak health systems. The recent renewal of this goal has received global support evidenced by an increase in international aid towards containing malaria transmission (Feachem and Sabot 2007). A recent major shift in the fight against malaria occurred in 2007, by changing goals from control to elimination and eventual eradication of malaria.

Key landmarks that preceded the current malaria eradication agenda:

- Global malaria control strategy (GMCS) was endorsed by a ministerial conference on malaria in 1992. The strategy was approved by the World Health Organization in 1993.
- Roll Back Malaria Partnership (RBM) was launched by the director general of WHO in 1998.
- The Abuja declaration 2000 was signed by heads of state of the African union, declaring to halve malaria mortality by 2010 by implementing the strategies and actions of RBM.
- Malaria became part of the millennium development goals, in consideration of the economic burden that the disease gives to endemic countries.
- Global fund for AIDS, TB and Malaria (GFATM) was established in 2002, as platform for providing financial support to aid the achievement of health related millennium development goals.
- Commitment to global malaria elimination was made in 2007 by Bill and Melinda Gates Foundation.
- Global malaria action plan was launched in 2008 by RBM partnership to act on the renewed goal of global malaria eradication.

Across much of sub Saharan Africa, the strategy is towards malaria control that is “reduction of disease incidence, prevalence, morbidity or mortality to a locally acceptable level as result of deliberate efforts” as opposed to elimination “reduction to zero of the incidence of locally transmitted malaria infection in a defined geographical area as a result of deliberate efforts”(Alonso, Brown et al. 2011). Here, a description is given of key malaria control measures based on either being preventive reducing the contact between humans and mosquitoes, therapeutic against those who are infected, or diagnostic to determine infection prevalence. The effectiveness of these interventions is likely to vary from one area to another depending on the intensity of malaria transmission and on the interactions with other interventions.

1.5.1 Prevention

Prevention of contact between mosquitoes and humans

Mosquito nets: Mosquito nets provide a physical barrier between humans and mosquitoes, in particular towards vectors that bite predominantly at night when most people are asleep (Lengeler 2004). Apart from offering a physical barrier, nets are embedded with synthetic insecticides of the pyrethroids class (permethrin or deltamethrin), which provide an additional chemical barrier. These insecticides can either kill mosquitoes that land on it or repel host-seeking mosquitoes, therefore increase the protective efficacy of insecticide impregnated bed nets as opposed to untreated nets. The fibres of Long Lasting Insecticide Treated Nets (LLINS) have the pyrethroids insecticide either embedded or bound to its fibres therefore increasing persistence. The insecticide in LLINs should remain active against mosquitoes even after at least 20 WHO standard washes, and after three years of use as opposed to insecticide treated bed nets that required re-impregnation after every three to six months (WHO 2007).

Mosquito nets offer personal protection from mosquito bites to the user, as well as to the community particularly when a large proportion (>60%) of the human population sleep under mosquito nets (Binka, Indome et al. 1998; Hawley, Phillips-Howard et al. 2003; Killeen, Smith et al. 2007). At large the mosquito population size may be affected due to reduction in the rate of survival and reduced chances of encountering hosts for a blood meal that is crucial for development of the next generation of mosquitoes. The reduction in mosquito survival reduces the chances of malaria parasite development inside the mosquito, for example *P. falciparum* under optimal conditions requires about 10 days inside the mosquito for it to become infectious. As a result of these combined effects findings from randomized trials of ITNs have associated use of ITNs with saving 5.5 lives per year for every 1000 children under 5 years of age protected as well as preventing clinical episodes of *P. falciparum* and *P. vivax* malaria by an average of 50% (range 39%-62%) (Lengeler 2004).

LLINs are one of the major vector control intervention currently being advocated by WHO for universal coverage across malaria endemic settings (WHO 2011). However, there may be emerging financial challenges on increasing access of this intervention as well as on replacing

worn out LLINs that are currently in use, ideally the replacement should be after 3-5 years (WHO 2007). Despite this challenge, there are reports from malaria endemic countries where physiological resistance by the mosquitoes towards insecticides including pyrethroids that are used in ITNs/LLIN have been reported (Ranson, N'Guessan et al. 2011). Such a situation raises an alert on reliance on pyrethroids and calls for development of alternative insecticides that can be safely used in bednets.

Indoor Residual Spraying (IRS): IRS is based on application of ideally long-lasting insecticides on walls and roofs of human habitation as well as animal shelters that are close to these human settlements (WHO 2006; Pluess, Tanser et al. 2010). There are four classes of insecticides that are being used for this purpose; oraganochlorides (Dichlorodiphenyltrichloroethane - DDT), organophosphates (e.g. Malathion, Fenitrothion), pyrethroids (e.g. Cyfluthrin, Deltamethrin) and carbamates (Bendiocarb & Propoxur) (WHO 2006). These insecticides can work by either repelling or by killing mosquitoes that come in contact with it upon landing on sprayed surfaces. Dichlorodiphenyltrichloroethane (DDT) and pyrethroids are well suited among these due to their reported lower vertebrate toxicity and long lasting residual effect (WHO 2006). Pyrethroids work by killing mosquitoes that come in direct contact with the insecticide, but at times it may be after mosquitoes have had a blood meal, but DDT repels them from entry into sprayed household, therefore reducing more the chances of human-mosquito contact. In general, mosquitoes that will not be repelled but make contact with either insecticide are more likely to be killed before they can bite another host. Eventually with IRS humans benefit from lowered rates of malaria transmission as a result of reduced contact, survival of mosquitoes as well as reduced effective size of mosquito population (Pluess, Tanser et al. 2010).

IRS was associated with the historical success of malaria elimination and eradication in the 1950s and 60s (Pluess, Tanser et al. 2010), the current challenge against IRS is the development of physiological as well as behavioural resistance among mosquitoes towards insecticides. The latter is reflected by the change in mosquito resting behaviour in the residual

mosquito population by reduced preference of resting indoors to increased preference towards resting outdoors.

Larval Source Management (LSM): This approach aims at reducing the population size of aquatic larval stages of mosquitoes by either reducing creation of favourable breeding habitats achieved by environmental engineering or by killing larvae based on use of chemical or biological larvicides (Soper and Wilson 1943; Fillinger and Lindsay 2011). Larvicides can be biological such as *Bacillus thuringiensis* (Bti) that work by releasing by-products that are toxic to mosquito larvae, by introduction of fish that can prey on mosquito larvae, by application of insect growth regulators that limit development of larvae into adult mosquitoes or addition of materials that form a layer at the surface of water as result lead to death of larvae by suffocation (Fillinger and Lindsay 2011; Raghavendra, Barik et al. 2011).

Currently the WHO recommends use of larviciding as a supplement to personal protection measures, LLINs and IRS and not as a stand alone intervention (WHO 2012). Since this intervention targets mosquitoes at their larval stage, it has no bias towards outdoors or indoor biting mosquitoes, a shortfall of the above mentioned indoor personal protection measures. Integration of LSM into these widely used personal protection measures can help to amplify the added value of these combined interventions in reducing malaria transmission. This integration of LSM into a setting has to be done in consideration of the local malaria transmission dynamics as well as the set up of local malaria control programme. LSM requires identification of breeding sites, devising ways to access them and allocation of resources both human and capital for its delivery and management. In areas where potential areas for malaria transmission e.g. swamps or identified hot spots are identified targeted LSM may be of great value.

In Africa, agriculture is practiced in both rural and urban areas, but more intensely in the former where it may be associated with large and permanent bodies of water that offer favourable conditions for breeding of anophelines. It is not easy to get estimates of the efficacy of this intervention based on the difficulty of designing randomized controlled trials for LSM,

but notable success stories have be en attributed to this approach of vector control (Soper and Wilson 1943; Shousha 1948).

Mosquito repellents: There are two types of repellents, plant-derived essential oils e.g. citronella and synthetic chemicals repellents e.g. N, N-diethyl-3-methylbenzamide -DEET, by reducing human-vector contact, repellents can also be considered as important supplementary tools against malaria transmission (Syed and Leal 2008; Maia and Moore 2011). The efficacy of repellents that use DEET as the main active ingredient are dependant on its concentration in the final product, higher concentration of DEET up to less that 50% are associated with up to four hours of protection to users, with an increment of one hour for DEET concentrations that are greater than 50%. Extended release formulations of DEET have been made that offer prolonged duration of protection without necessarily increasing the concentration of DEET. Plant derived essential oils offer up to two hours of protection (Maia and Moore 2011). Decisions of how to integrate repellents as personal protection measures or as spatial repellents may be dependent on the feeding and resting behaviour of local mosquito population. Repellents can have a wide scale application when embedded into materials such as mats, and more recently as durable wall linings (Messenger, Miller et al. 2012) suitable to be hung inside houses where there are endophilic mosquitoes.

House screening: Proofing indigenous houses against entry of mosquitoes by screening windows, doors, eaves, and installing ceiling boards will reduce the proportion of mosquitoes that enter indoors (Lindsay, Emerson et al. 2002; Kirby, Ameh et al. 2009). Mosquitoes seek cool shelters during the heat of the day typical across malaria endemic areas, since their bodies are sensitive to temperature fluctuations and are prone to desiccation at high temperature. They find entry through opening such as eaves, windows, doors at night. Eaves are associated with a higher mosquito entry since *An. gambiae* tend to fly upwards upon encountering an obstacle such as a wall, therefore gaining access through eaves (Lindsay, Emerson et al. 2002). Grass thatching offers an even more suitable hiding place for anophelines than iron sheet roofs. Female anophelines exit from resting indoors in the evening to seek a mate, thereafter return indoors to host seek as a result transmit malaria. The mosquito population will be

affected by reduction in the chance of getting a blood meal necessary for development of eggs as well as by reducing the proportion of favourable resting sites for highly endophilic mosquitoes. Screening of these mosquito gateways reduces the chances of malaria transmission from indoor biting mosquitoes to all household members. This intervention can be integrated into existing interventions but also form a part of existing development programs that are aiming at improving the wellbeing of people in malaria endemic settings. In addition this approach not only improves house design but also increase aesthetic value of houses.

House position: Mosquitoes are limited on the range upon which they can host seek by the distance upon which they can cover by flight, different studies give different estimates but it has been reported to range from 1 to 5 km (WHO 1975; Costantini, Li et al. 1996). Discouragement of human habitation beyond this range may reduce the risk of malaria transmission. Irrigation schemes, river flood plains are an example of areas that are associated with intense malaria transmission. This approach may form part of human settlement plans of areas endemic to malaria transmission.

Zooprophylaxis: Is the practice of keeping domestic animals near human settlements. Animals offer protection by offering an alternative source of blood for host seeking mosquitoes. This intervention may work best in areas where malaria transmission is maintained by mosquitoes such as *An. arabiensis* that have shown preference for both human and domestic animal's blood (Tirados, Costantini et al. 2006). Keeping animals inside houses may increase the number of mosquitoes that rest and host seek indoors, therefore reducing the protective efficacy of zooprophylaxis against humans as some fraction of mosquitoes may end up biting humans. Zooprophylaxis can supplement ITNs/LLINs for added benefits of human protection against malaria transmission.

Prevention of infection

Intermittent Preventive Treatment (IPT): IPT is based on administration of a therapeutic dose of an anti malarial to treat existing infections and prevent further malaria episodes in pregnant

woman (IPTp) and infants (IPTi) living in high risk of malaria transmission. IPTp is administered through the antenatal care services while IPTi is to be administered through the routine immunization programmes at 10 weeks, 14 weeks and 9 months. Sulphadoxine pyrimethamine (SP) is the drug that is used across all categories.

Prophylaxis: use of chemotherapy by for malaria naïve individuals prior to visiting malaria endemic settings can be protective by offering a form of partial immunity. Several drugs, most of which are used for treatment of malaria, can be taken for this purpose such as Mefloquine, or the combination of Atovaquone and proguanil hydrochloride (Malarone).

1.5.2 Treatment

The current most effective strategy for treatment of malaria is the use of artemisinin based combination therapy. The artemisin component of the combined therapy is fast acting against malaria parasites, is partnered with longer acting anti-malarials such as amodiaquine, lumefantrine, mefloquine or sulfadoxine pyrimethamine to prolong the fight against malaria parasites. Recent studies in the Thai-Cambodia border have reported signs of reduced susceptibility of *P. falciparum* to artemisins (Lim, Wongsrichanalai et al. 2008; Klein 2013), but efforts are underway to contain the spread of these parasite strains.

1.5.3 Diagnosis

Rapid and accurate diagnosis of malaria is important in providing appropriate and timely treatment. Rapid diagnostic tests for malaria are currently being rolled out to supplement the diagnosis based on microscopy. WHO recommends that all suspected malaria cases should be confirmed by diagnosis of the malaria parasite by either microscopy or RDTs before receiving malaria treatment.

1.5 Study platform

The MTIMBA (Malaria Transmission Intensity and Mortality Burden across Africa)

The MTIMBA project was a multi-centre study with sites from West, East and Southern Africa. The study design included eighteen malaria-endemic sites in Africa, the analyses presented in this thesis utilized MTIMBA data from Tanzania (Rufiji and Kilombero-Ulanga) as well as Burkina Faso (Nouna, Oubritenga and Kourweogo). It was carried out between the years 2001 and 2004. The aim of this study was to measure all cause and malaria specific mortality in relation to malaria transmission intensity, while considering malaria contextual factors that might influence the relationship between malaria transmission and mortality.

- All cause mortality was monitored as part of ongoing demographic surveillance system (DSS).
- Malaria cause-specific mortality was estimated using a standardized verbal autopsy.
- Entomological methods on sampling adult host seeking mosquitoes were harmonized between sites.

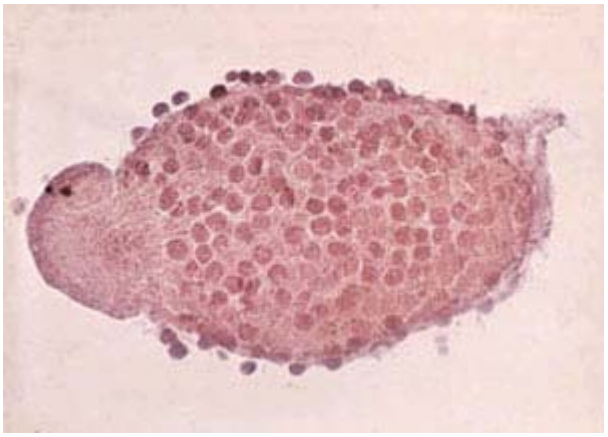
The project received funding from The Special Programme for Research and Training in Tropical Diseases Research (MIM/TDR) and the Roll Back Malaria Programme (RBM).

MONITORING AND EVALUATION TOOLS TO ALLOW SUSTAINED ELIMINATION OF MALARIA TRANSMISSION

A study under the Malaria Transmission Consortium (MTC) aimed at developing and characterizing a new set of surveillance tools that enable sustained elimination of malaria transmission through constant monitoring, evaluation and adaptation of integrated control programmes, suitable for even low transmission scenarios where malaria is approaching elimination. The study was carried out between 2009 and 2010 and included this thesis are data from Zambia and Kenya. Funding for this study was received from the Bill & Melinda Gated Foundation. ,

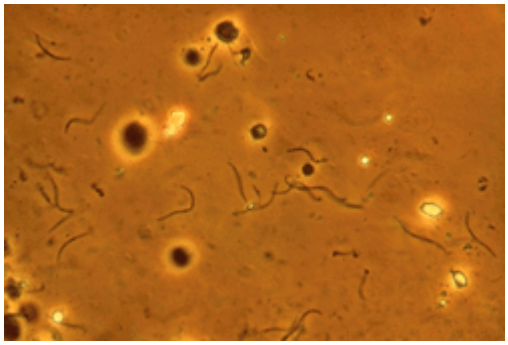
1.6 Justification

Roll Back Malaria aimed to reduce the burden of malaria so that by the year 2030 malaria would cease to be a public health problem. To achieve this goal relied not only on rolling out appropriate interventions such as insecticide treated materials, but also on monitoring the trends in malaria transmission. This required collection of reliable data on both the vectors and human populations in endemic countries at baseline and other time points. Entomological information such as vector species and density, proportion of infected mosquitoes and the inoculation rate as key transmission parameters was essential to the understanding of the epidemiology of malaria and for planning of control measures. The study platforms described in this thesis were essential in answering questions at a large scale on different aspects of vector behaviour and tools used to estimate their population dynamics. Furthermore, an assessment of the impact of presence or lack of large scale clinical treatment of malaria on human infectiousness was assessed on two sites that had intense malaria transmission intensity. Baseline measures obtained are important in understanding the relationship between malaria transmission intensity and mortality in these areas while considering vector mediated transmission intensity as well as other post inoculation factors measured in associated studies that including ccess to anti-malarial drugs.



A mosquito gut that is heavily infected with *Plasmodium* oocysts mosquito gut

(Source:http://en.impact-malaria.com/web/malaria_training/human-vector_transmission)



Sporozoites observed in the mosquito salivary glands after dissection.

(Source: http://en.impact-malaria.com/web/malaria_training/sexual_stages/sporogony)

Artemisinin-based combination therapy does not measurably reduce human infectiousness to vectors in a setting of intense malaria transmission

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2. Artemisinin-based combination therapy does not measurably reduce human infectiousness to vectors in a setting of intense malaria transmission

2.1 Abstract

Background

Artemisinin-based combination therapy (ACT) for treating malaria has activity against immature gametocytes. In theory, this property may complement the effect of terminating otherwise lengthy malaria infections and reducing the parasite reservoir in the human population that can infect vector mosquitoes. However, this has never been verified at a population level in a setting with intense transmission, where chronically infectious asymptomatic carriers are common and cured patients are rapidly and repeatedly re-infected.

Methods

From 2001 to 2004, malaria vector densities were monitored using light traps in three Tanzanian districts. Mosquitoes were dissected to determine parous and oocyst rates. *Plasmodium falciparum* sporozoite rates were determined by ELISA. Sulphadoxine-pyrimethamine (SP) monotherapy was used for treatment of uncomplicated malaria in the contiguous districts of Kilombero and Ulanga throughout this period. In Rufiji district, the standard drug was changed to artesunate co-administered with SP (AS+SP) in March 2003. The effects of this change in case management on malaria parasite infection in the vectors were analysed.

Results

Plasmodium falciparum entomological inoculation rates exceeded 300 infective bites per person per year at both sites over the whole period. The introduction of AS+SP in Rufiji was associated with increased oocyst prevalence (OR [95%CI] = 3.9 [2.9-5.3], $p < 0.001$), but had no consistent effect on sporozoite prevalence (OR [95%CI] = 0.9 [0.7-1.2], $p = 0.5$). The estimated infectiousness of the human population in Rufiji was very low prior to the change in drug

policy. Emergence rates and parous rates of the vectors varied substantially throughout the study period, which affected estimates of infectiousness. The latter consequently cannot be explained by the change in drug policy.

Conclusions

In high perennial transmission settings, only a small proportion of infections in humans are symptomatic or treated, so case management with ACT may have little impact on overall infectiousness of the human population. Variations in infection levels in vectors largely depend on the age distribution of the mosquito population. Benefits of ACT in suppressing transmission are more likely to be evident where transmission is already low or effective vector control is widely implemented.

2.2 Background

Currently, artemisinin-based combination therapy (ACT) is used as first-line treatment of uncomplicated malaria in most countries in sub-Saharan Africa. In addition to killing the asexual blood stages that cause disease and, therefore, terminating otherwise lengthy, persistently transmissible infections (Jeffery and Eyles 1955; Bruce, Donnelly et al. 2000; Sama, Owusu-Agyei et al. 2005), artemisinins are gametocytocidal, killing the immature sexual stages of malaria parasites eventually responsible for infecting mosquitoes (Nosten, van Vugt et al. 2000; Ashley and White 2005). While non-gametocyctocidal drugs will also cure otherwise lengthy infections and reduce the period of infectiousness to mosquitoes, gametocytes will remain in the cured individual for some time, allowing for transmission.

In principle, through their combined impacts upon both the short-term infectiousness of treated individuals, and perhaps more importantly (Okell, Drakeley et al. 2008), upon the long-term duration of infection and therefore infectiousness, ACT might reduce the reservoir of parasites in the human population that eventually infects mosquitoes.

The provision of ACT for treatment of uncomplicated malaria has been associated with reduced malaria incidence in diverse settings with modest transmission intensity (Nosten, van Vugt et al. 2000; Barnes, Durrheim et al. 2005; Bhattarai, Ali et al. 2007). This implies that ACT may effectively reduce human-to-mosquito and consequently mosquito-to-human transmission under normal conditions of programmatic use, as has been suggested in individually randomized, controlled trials evaluating the infectiousness of patients receiving ACT (Drakeley, Jawara et al. 2004; Sutherland, Ord et al. 2005; Barnes, Chanda et al. 2009).

Determination of the proportion of humans harbouring gametocytes following ACT treatment may not accurately estimate human population infectiousness since infectiousness seems only loosely correlated to gametocyte density (Graves, Burkot et al. 1988; Haji, Smith et al. 1996). In malaria-endemic settings, humans can be infectious to mosquitoes even in the absence of patent gametocytaemia, regardless of treatment (Jeffery and Eyles 1955; Bousema, Gouagna et al. 2004; Bousema, Schneider et al. 2006; Schneider, Bousema et al. 2006). While human-to-mosquito feeding experiments with laboratory-reared mosquitoes are very useful, they do not

capture parasite infection and selection dynamics in the context of their human host populations (Ferguson, Rivero et al. 2003; Mackinnon and Read 2003; de Roode, Pansini et al. 2005; Okell, Drakeley et al. 2008) and are not necessarily representative of the wild mosquito populations which have natural feeding biases influenced by host age and infection status (Graves, Burkot et al. 1990; Lacroix, Mukabana et al. 2005; Ross, Killeen et al. 2006; Mukabana, Takken et al. 2007). Estimation of the human infectious reservoir therefore requires analysis of the infection status of wild-caught mosquitoes.

A pre-post observational study with a contemporaneous comparison group was used to evaluate the impact of case management with ACT delivered through fixed health facilities in two sites in rural Tanzania with intense malaria transmission (Khatib, Skarbinski et al. 2012). Both the intervention and comparison sites used sulphadoxine-pyrimethamine (SP) as first-line treatment of malaria in 2001–2003. In March 2003, the ACT, artesunate co-administered with SP (AS+SP), was introduced as a first-line treatment of malaria in the intervention site while SP continued to be used for first-line treatment in the comparison site. To assess the impact of ACT introduction on malaria transmission, concurrent measures of oocyst and sporozoite prevalence in the mosquito-vector population in both the intervention and comparison districts, before and after the introduction of AS+SP, were carried out and used to directly determine the infectiousness of the human population to mosquitoes, and of mosquitoes to humans.

2.3 Methods

2.3.1 Study site

This study was conducted in two rural sites in southeastern Tanzania. Rufiji District, the intervention site, is located at the mouth of the Rufiji River, extends across latitudes 7° 47′ and 8° 03′S and longitudes 38° 62′ and 39° 17′E with a population of about 202,001 inhabitants (National Bureau of Statistics 2003; Mwageni, Masanja et al. 2005). Kilombero and Ulanga Districts, the comparison site, form the valley of the Kilombero River, one of the main

tributaries of the Rufiji and are situated between latitudes 8°00'–8°35'S, longitudes 35°58'–36°48'E and have a combined population of 514,891 inhabitants (Armstrong-Schellenberg, Mukasa et al. 2002; National Bureau of Statistics 2003) (Figure 1). Both Rufiji and Kilombero-Ulanga Districts have achieved relatively high coverage of largely untreated bed nets (Killeen, Tami et al. 2007; Khatib, Killeen et al. 2008) and are characterized by a hot climate with an erratic rainy season from November to May. In Rufiji, the average annual precipitation is 800-1,000 mm while Kilombero-Ulanga receives 1,200-1,800 mm. In both settings, malaria caused largely by *Plasmodium falciparum* (Abdullah, Adazu et al. 2007) is one of the biggest health problems perceived by the local community and reported by the health services (Mwageni, Momburi et al. 2002). It is primarily transmitted by *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles funestus*. Transmission is intense and perennial despite marked seasonality in mosquito densities, which peak with the rains (Schellenberg, Menendez et al. 2004; Abdullah, Adazu et al. 2007).

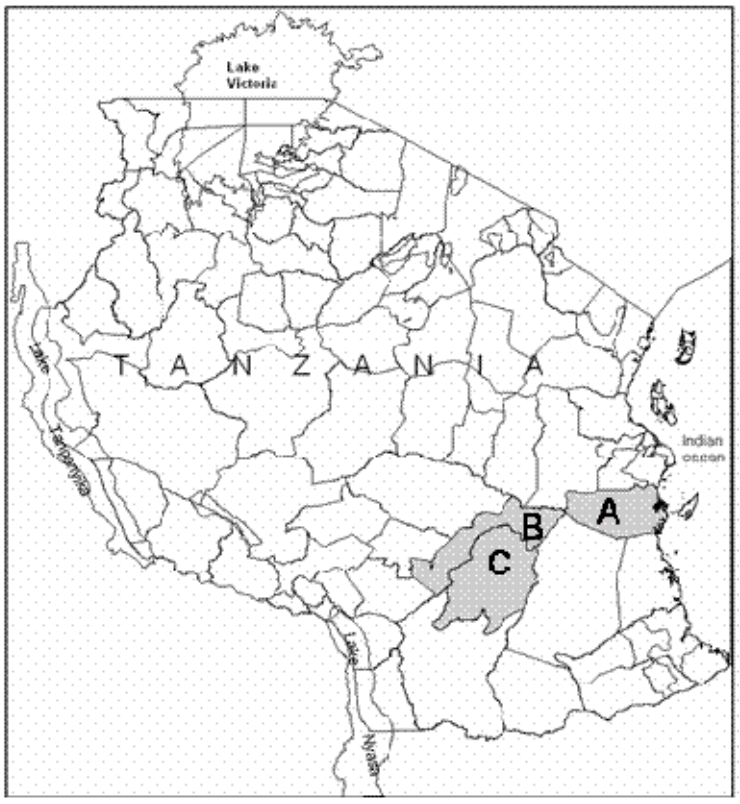

Figure 1 Map of the study districts. A: Rufiji; B: Kilombero; C: Ulanga.

2.3.2 Study design

The detailed description of the study is reported elsewhere (Khatib, Skarbinski et al. 2012). Briefly, a pre-post observational study with a non-randomized comparison site was conducted. Both sites used SP monotherapy as a first-line anti-malarial delivered through health facilities from 2001. In March 2003, the Council Health Management Team implemented AS+SP combination therapy as the first-line anti-malarial provided free of charge through all the fixed health facilities in Rufiji District, the intervention site. SP monotherapy continued to be the first-line anti-malarial in Kilombero-Ulanga, the comparison site, as well as in the rest of the country. Here, SP was available free of charge only to pregnant women and children under five years of age.

2.3.3. Mosquito data collection

In each site, anopheline indoor biting rates were determined by overnight trapping of host-seeking mosquitoes using Centers for Disease Control and Prevention (CDC) light traps. The two sites used slightly different household selection procedures. Sampling in Kilombero-Ulanga occurred from October 2001 to August 2004 and was based on repeated sampling every six months of 25 clusters of households selected by stratified random sampling, using the sub-village (*kitongoji*) as the first level and index household as the second level of randomization (Killeen, Tami et al. 2007). Trapping was carried out on 538 different nights, with an average of 4.9 traps per night. The traps were clustered in houses around the index house, but out of sight of each other.

In Rufiji, the period sampled included a 17-month pre-intervention period of October 2001 to February 2003 and a 19-month post-intervention period of March 2003 to September 2004. Individual households were randomly selected monthly from the same demographic surveillance sample frame used for surveys of human malaria infection (Khatib, Skarbinski et al. 2012). Trapping was carried out on 850 distinct dates, with an average of 6.6 traps per night.

Light traps were installed about 0.5 m above the floor, next to the foot of the bed of the selected person who slept under a mosquito net. No attempt was made to differentiate

between treated and untreated nets in the field as this proved impractical during routine field surveys and insecticide treatment has only a minor effect on sampling efficiency (Magbity, Lines et al. 2002; Killeen, Tami et al. 2007). On occasions when the selected individual for light trap sampling lacked a net, he or she was provided with an untreated net for the nights during which they participated.

Once collected, mosquitoes were counted and sorted by species in the field. Where this was feasible, blood-fed female *An. gambiae* s.l. and *An. funestus* were held in a cup and fed on sugar water until the blood meal was digested, this period ranges from two to three days depending on temperature. Then, the mid-guts of these mosquitoes were dissected in normal saline and stained with 2% mercurochrome for examination of oocysts by light microscopy (Haji, Smith et al. 1996). The remaining parts of the dissected mosquitoes as well as other undissected anophelines were routinely stored in Eppendorf tubes with a small quantity of silica gel. Mosquitoes were subsequently independently tested for circumsporozoite protein (CSP) by ELISA (Burkot, Williams et al. 1984) in a central laboratory at Ifakara Health Institute. At each site, a different technician conducted the mosquito dissections and examinations for the presence of oocysts. Laboratory technicians performing the CSP ELISA were blinded to the oocyst status and source of the mosquitoes to avoid possible biases in the determination of sporozoite infection status.

2.3.4 Ethical approval

Ethical approval was obtained from the Medical Research Coordination Committee of the National Medical Research Coordination Committee of National Institute for Medical Research, Tanzania (Reference number NIMR/HQ/R.8a/VOL.VIII, dated April 2000).

2.3.5 Data analysis

The overall objective of the analysis was to determine the relationship between the introduction of ACT and the infectiousness of the human population, as reflected by infection prevalence in local vector populations. The outcome measures reflecting human-to-mosquito transmission were the infection status of individual mosquitoes, with the primary and secondary effects defined by the presence of oocysts or sporozoites, respectively, within the two study zones. The proportions of mosquitoes with oocysts and sporozoites (the oocyst and sporozoite rates, respectively) were estimated independently for groups of mosquitoes collected before and after the introduction of ACT in the intervention site. Multivariate logistic regression models with terms for study site (intervention versus comparison), period of mosquito collection (pre-intervention versus post-intervention), intervention (availability of ACT versus SP monotherapy), and species of mosquito (*An. gambiae* s.l. versus *An. funestus*), were used to assess the impact of the introduction of ACT on oocyst and sporozoite prevalence. Statistical significance was defined as a p-value ≤0.05. All statistical analyses were executed using SPSS 15.0 (SPSS Inc, Chicago, USA).

To measure mosquito-to-human malaria transmission intensity, the entomological inoculation rate (EIR) was calculated by multiplying the arithmetic mean mosquito-biting rate per night by the mean sporozoite prevalence for that vector species. EIR was calculated separately for the pre- and post- intervention periods. The biting rate for each mosquito species was obtained by dividing the mean catch of females in CDC light traps by published estimates from the Kilombero Valley of the relative sensitivity of CDC light traps relative to human landing catches of 0.30 and 0.68 for *An. gambiae* s.l. and *An. funestus*, respectively (Okumu, Kotas et al. 2008).

Infectiousness of humans to mosquitoes depends on K, the proportion of mosquitoes that are infected at any given feed. This cannot be measured directly, because infected mosquitoes may have received their infections either at the latest, or at a previous feed. There are various algorithms for estimating K from field-caught mosquitoes. All of these require both a measure of the proportions of mosquitoes that are infected, and a measure of the age distribution of

the vectors. For the present study, K was estimated from the proportions of host-seeking mosquitoes with oocysts and the proportion that were parous using the following equation (Charlwood, Smith et al. 1997; Killeen, Ross et al. 2006):

$$K_O = \frac{1 - 1/M}{1 - M/R}$$

Where: *M* is the proportion of parous mosquitoes among those dissected and *R* is the proportion of dissected mosquitoes with oocysts (the immediate oocyst rate). The standard error of K_O was determined as described previously (Charlwood, Smith et al. 1997).

3 Results

In Rufiji, 11,883 *An. gambiae* s.l. and 13,434 *An. funestus* were sampled before ACT introduction, while 5,826 *An. gambiae* s.l. and 2,626 *An. funestus* were sampled after ACT introduction. In the comparison site: Kilombero-Ulanga, 50,694 *An. gambiae* s.l. and 9,615 *An. funestus* were sampled before and 27,559 *An. gambiae* s.l. and 8,381 *An. funestus* after ACT introduction in Rufiji. The density of anophelines as well as the parous rate varied seasonally and strongly between years (Figures 2 and 3). Fewer mosquitoes were caught post the intervention in Rufiji, but both 2003 and 2004 were very dry years (Figure 4) and this was presumably the main factor affecting mosquito densities.

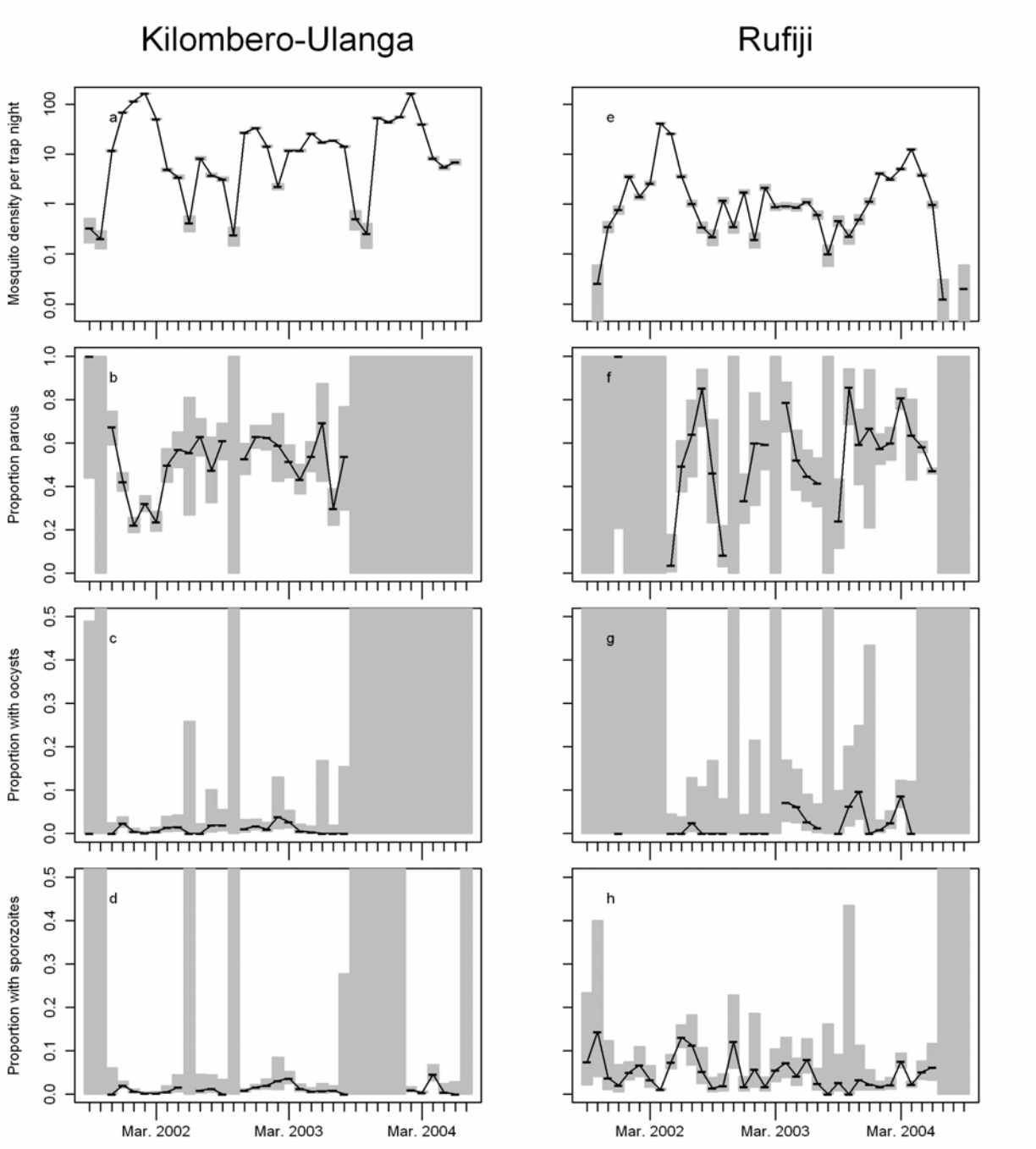

Figure 2 *Anopheles gambiae* s.l. density (panels a and e), proportion parous (panels b and f), proportion with oocysts (panels c and f) and proportion with sporozoites (panels d and g) for Kilombero-Ulanga (panels a – d) and Rufiji (panels e – g) districts by month. Horizontal black lines represent observed values, grey bars represent 95% confidence intervals. Subsequent non-missing values are connected by thin black lines.

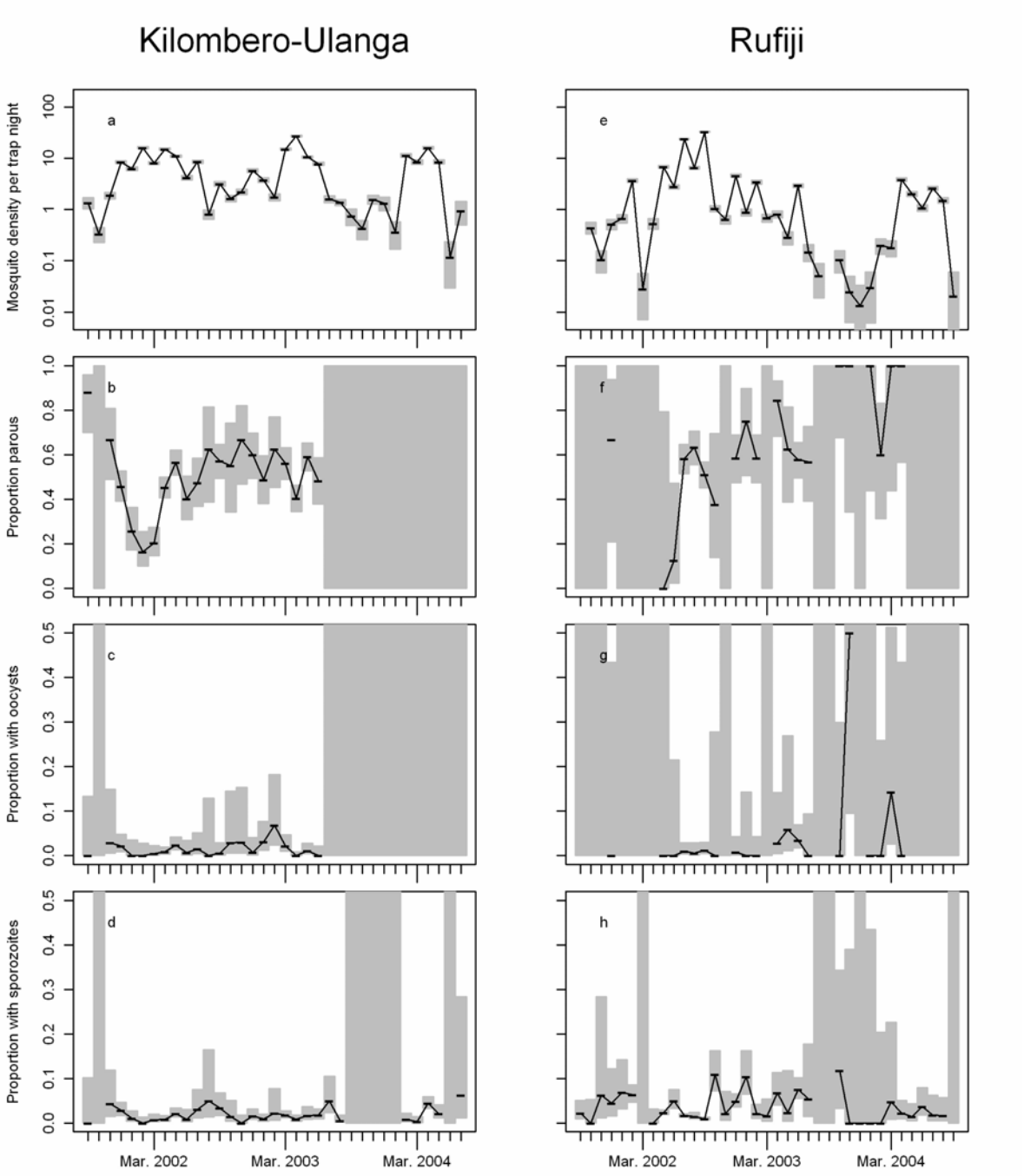

Figure 3 *Anopheles funestus* density (panels a and e), proportion parous (panels b and f), proportion with oocysts (panels c and f) and proportion with sporozoites (panels d and g) for Kilombero-Ulanga (panels a – d) and Rufiji (panels e – g) districts by month. Horizontal black lines represent observed values, grey bars represent 95% confidence intervals. Subsequent non-missing values are connected by thin black lines.

Oocyst prevalence in Rufiji increased substantially between the pre-intervention and post-intervention period (χ^2 = 11.9, p <0.001 for *An. gambiae*, χ^2=11.1, p <0.001 for *An. funestus*) with an odds ratio (estimated from a multivariable logistic regression, allowing for site, species, and time period) of 3.9 [95%CI: 2.9-5.3] (Figure 5). However, the confidence intervals for both *An. funestus* and *An. gambiae* s.l. oocyst rates were wide (Figures 2 and 3 respectively) because of the considerable inter-month variation. No significant changes (χ^2 = 0.01, p =0.9 for *An. gambiae*, χ^2=0.04, p = 0.8 for *An. funestus*) were observed in Kilombero-Ulanga (Table 1, Figures 2-4). Sporozoite prevalence also increased significantly in Rufiji for *An. funestus* (χ^2=37.3, p<0.001), but not for *An*. *gambiae* s.l. (χ^2 = 0.02, p =0.9) so overall there was little effect (OR [95%CI] = 0.9[0.7-1.2], p = 0.51) (Table 2) while in Kilombero-Ulanga the sporozoite prevalence increased significantly for *An*. *gambiae* s.l. (χ^2 = 21.6, p <0.001), but not for *An. funestus* (χ^2=1.7, p =0.19). These formal statistical comparisons between pre- and post-intervention periods must be viewed cautiously in the context of the considerable seasonal and inter-annual variation in both mosquito densities, and in the numbers of mosquitoes that were analysed for each outcome. The age distribution of the mosquito populations, as indicated by the parous rates, also varied considerably over time, reflecting variations in both mosquito survival and recruitment rate to the vector populations. Environmental variation (Figure 4) is probably the main determinant of longitudinal patterns in mosquito bionomics. Because of the profound inter-annual differences we did not attempt to adjust these analyses for seasonality.

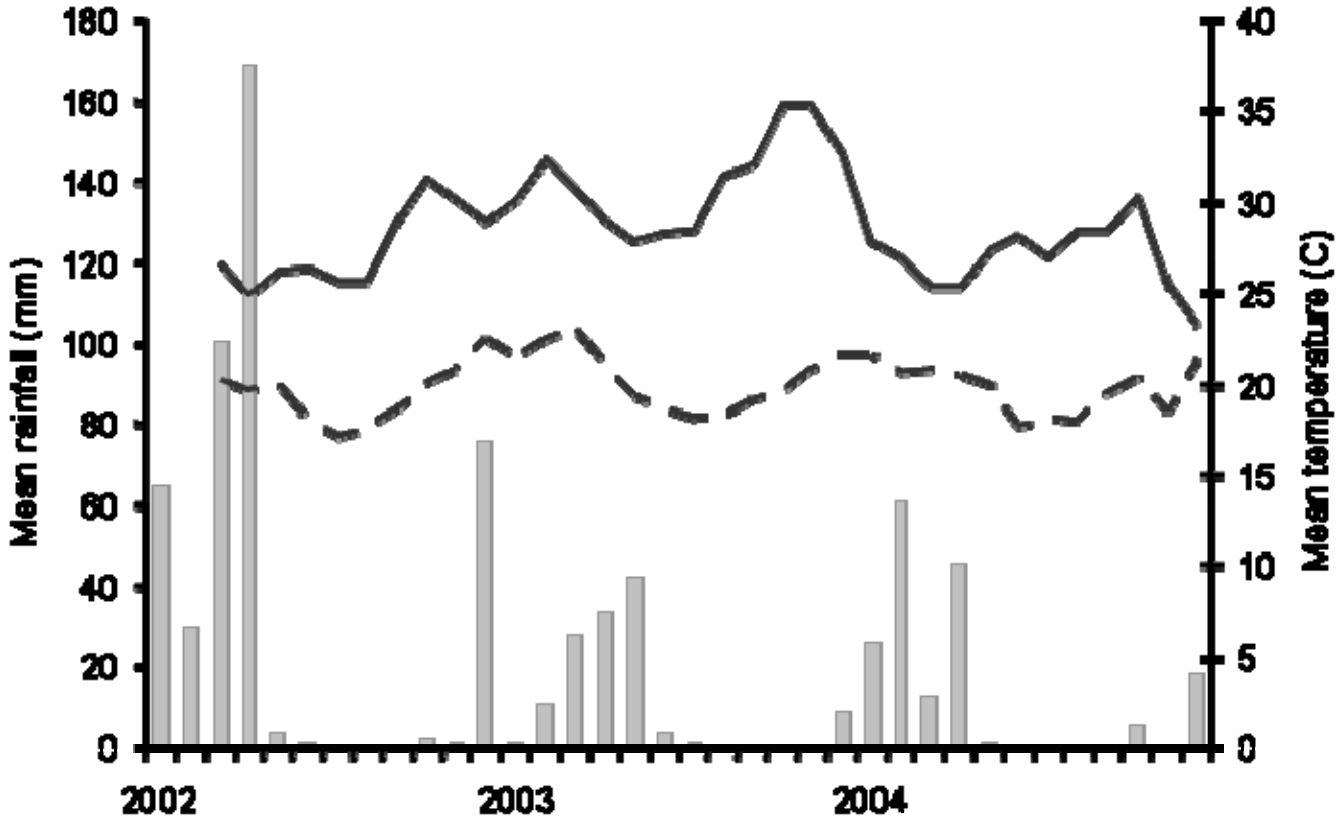

Figure 4 Temperature and rainfall for Rufiji for the period of 2002-2004. The bars represent the rainfall per month (left axis), the lines show the monthly maximum (solid line) and minimum temperature (broken line), right axis. Values are based on remote sensing. Rainfall data were obtained from the Africa Data Dissemination Service (*ADDS*) (USGS) and temperature data from the National Aeronautics and Space Administration (NASA).

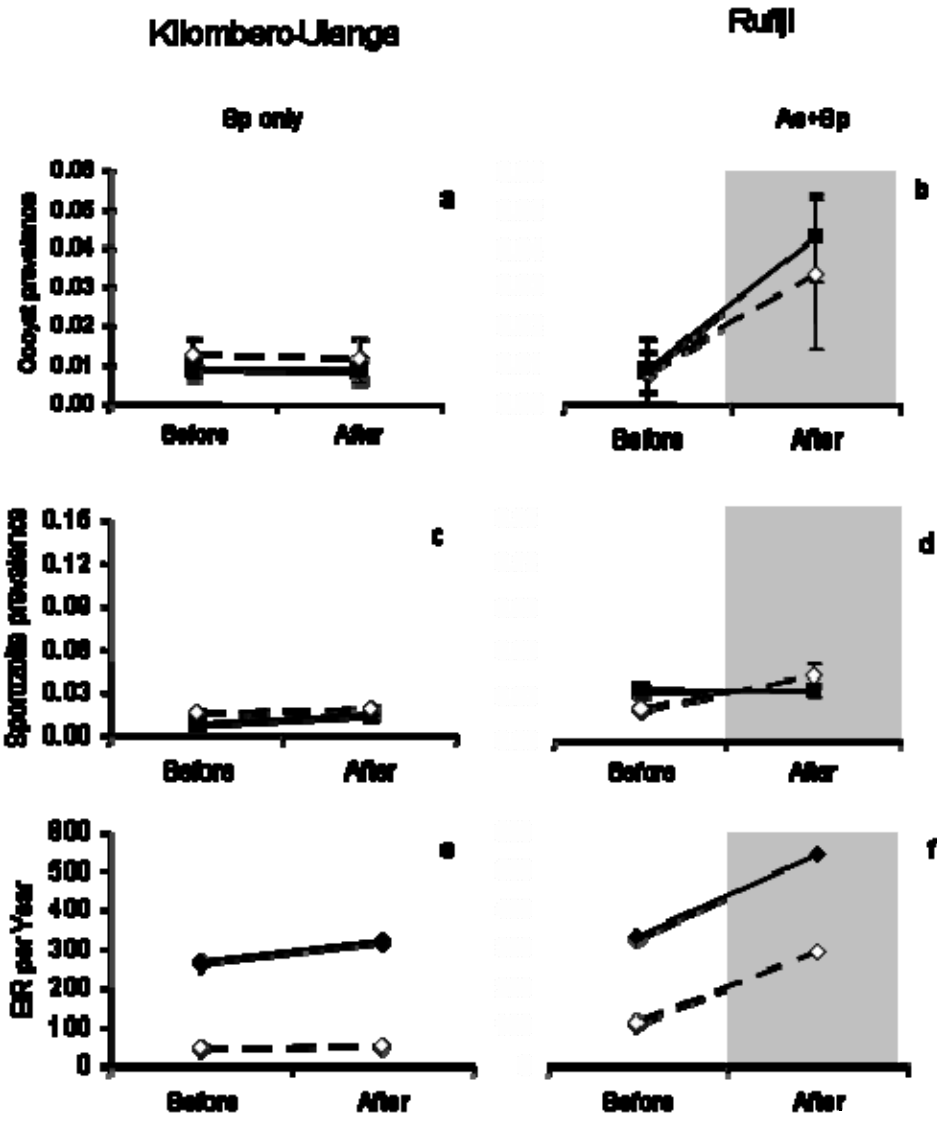

Figure 5 Trends in mosquito infection prevalence. Observed trends of mosquito oocyst (A & B) and sporozoite (C & D) prevalence before and after the onset of an artesunate-based effectiveness trial, error bars correspond to 95% confidence interval. A comparison can be made for *Anopheles gambiae* s.l. (straight line and dark squares) and *Anopheles funestus* (dotted line, white squares). Panels E & F show the trends in malaria transmission. The shading on the graphs serves to indicate the transition from before and after the addition of AS to SP.

Mosquito-to-human transmission, as estimated by the EIR, consistently exceeded 300 infective bites per person per year in both sites throughout the study period (Table 1). During both the pre-intervention and post-intervention time periods, the intervention site had the highest proportion of sporozoite-positive mosquitoes, and therefore the highest EIR. The estimated EIR for both *An. gambiae* s.l. and *An. funestus* in the intervention site was higher after ACT introduction than before. This coincided with a possible increase in human-to-mosquito transmission implied by the observed increase in oocyst prevalence. In the absence of an increase in prevalence of sporozoites in *An. gambiae* s.l. it is impossible to draw any firm conclusions about effects on the infectious reservoir, which does not necessarily follow the EIR in endemic settings (Killeen, Ross et al. 2006). One clear conclusion though is that the introduction of ACT was not followed by signs of a decline in human-mosquito transmission.

The estimates of infectiousness of the human population were summarized at the level of the time period (pre- or post-policy change), by site, and by vector species (Table 1). The values of K_O were similar for both vector species, both sites and both time periods, with the exception of the pre-intervention values for Rufiji, which were very low. Much of the variation in sporozoite and oocyst prevalence can thus be attributed to variations in mosquito survival, which are accounted for by the term for the parous rate (*M*) in the formula for K_O.

The values estimated for K in the literature are extremely variable (Killeen, Ross et al. 2006) but few of them are as low as the values measured pre-intervention in Rufiji. The values for Kilombero-Ulanga do not show any indication of a trend over time, and are higher than the pre-intervention Rufiji ones, suggesting that the low values cannot be attributed to the use of SP as treatment. There is no indication that the post-policy change values for Rufiji reduced *K* below the Kilombero value.

Table 1 Prevalence of mosquitoes infected with oocysts and sporozoites and entomological inoculation rate (EIR) in Rufiji and Kilombero-Ulanga Districts by Anopheline species and time period

	Time period (Anti-malarial in use)	Oocyst prevalence		Sporozoite prevalence		EIR
		n/N	% [95% CI]	n/N	% [95% CI]	
Rufiji						
An. funestus	January 2002-February 2003 (SP)	9/1094	0.82 [0.29-1.36]	321/14861	0.022 [0.019-0.024]	108
	March 2003- September 2004 (AS+SP)	11/330	3.33 [1.40-5.27]	99/2273	0.044 [0.035-0.052]	288
An. gambiae s.l	January 2002-February 2003 (SP)	4/475	0.84 [0.02-1.66]	291/8665	0.034 [0.030-0.037]	332
	March 2003-September 2004 (AS+SP)	51/1195	4.27 [3.12-5.41]	215/6475	0.033 [0.029-0.038]	538
Kilombero-Ulanga						
An. funestus	January 2002-February 2003 (SP)	31/2518	1.23 [0.80-1.66]	63/4353	0.014 [0.011-0.018]	45
	March 2003- August 2004 (SP)	21/1806	1.16 [0.67-1.66]	117/6576	0.018 [0.015-0.021]	50
An. gambiae s.l	January 2002-February 2003 (SP)	40/4506	0.89 [0.61-1.16]	63/9333	0.007 [0.005-0.008]	267
	March 2003- August 2004 (SP)	24/2765	0.87 [0.52-1.21]	128/9372	0.014 [0.011-0.016]	320

EIR=Entomological inoculation rate expressed as infectious mosquito bites per person per year

95% CI= 95% confidence interval

SP= Sulphadoxine-pyrimethamine; AS+SP= Artesunate co-administered with sulphadoxine-pyrimethamine

Table 2 Factors associated oocyst and sporozoite prevalence in Anopheline vectors in Rufiji and Kilombero-Ulanga Districts, January 2002-July 2004

Variable	Oocyst prevalence		Sporozoite prevalence	
	OR [95% CI]	P value	OR [95% CI]	P value
District				
Kilombero-Ulanga	Referent	Referent	Referent	Referent
Rufiji	0.72 [0.38-1.37]	0.31	2.51 [2.22-2.84]	<0.001
Period				
January 2002-February 2003	Referent	Referent	Referent	Referent
March 2003-July 2004	1.09 [0.76-1.58]	0.63	1.44 [1.28-1.61]	<0.001
Anti-malarial in use				
SP	Referent	Referent	Referent	Referent
AS+SP	3.91 [2.88-5.33]	<0.001	0.92 [0.72-1.18]	0.51
Anopheline species				
An. gambiae s.l	Referent	Referent	Referent	Referent
An. funestus	1.19 [0.88-1.61]	0.26	0.96 [0.85-1.07]	0.45

4. Discussion

Despite numerous clinical studies demonstrating high cure rates and gametocytocidal effect of artemisinin derivatives (Drakeley, Jawara et al. 2004; Falade, Makanga et al. 2005; Sutherland, Ord et al. 2005; Yeka, Banek et al. 2005), there is no evidence that this translates into any measurable impact on malaria transmission intensity at the population level in these Tanzanian sites. Although, the potential to reduce malaria transmission is widely cited, some mathematical models predict only a modest incremental impact of the routine use of ACT over non-gametocytocidal drugs in high transmission settings (Okell, Drakeley et al. 2008). This observational study of the impact of routine delivery of ACT via health facilities provides some empirical support for this. Although, the parasitological study found a significant reduction in asexual parasitaemia prevalence following ACT introduction, this reduction was very modest (five percentage-points) and was not reflected in a measurable reduction of gametocytaemia prevalence in the human population (Khatib, Skarbinski et al. 2012). In the present study, the most direct indicator of human-to-mosquito transmission, namely oocyst prevalence, was substantially higher after ACT introduction. It is unclear what caused this increase, particularly since the sporozoite prevalence did not increase at the same time (Table 2), only factors, in particular weather patterns (Figure 4) changed considerably between the two periods. Because environmental conditions and availability of mosquitoes for analysis varied erratically throughout the study period, it is not possible to formally separate inter-annual and seasonal variation from effects of the policy change, but the overall conclusion is that any ACT-related reductions in human-to-mosquito or mosquito-to-human transmission in the mosquito population were small.

Overall, these two large-scale, complementary studies of malaria parasite prevalence in both humans and mosquitoes did not detect any epidemiologically meaningful suppression of human population infectiousness following ACT introduction. However, mosquito population dynamics in Rufiji were clearly profoundly affected by variations in rainfall during the study period. Rainfall affects both the emergence rates of vectors, and probably (via effects on humidity) the survival of adult mosquitoes. This does not directly affect the infectiousness of

the human population to mosquitoes, but has profound effects on malaria transmission as measured either by the EIR or the oocyst prevalence. The large variations in emergence rates and survival of mosquitoes very likely account for most of the variation in oocyst prevalence, though this cannot explain why infectiousness was so low during the first half of the study (prior to ACT) in Rufiji, or why the oocyst prevalence increased after ACT introduction, while sporozoite prevalence did not. Far fewer mosquitoes were examined for oocysts than sporozoites, and sampling variation thus contributes more to the oocyst data.

The increase in oocyst prevalence thus seems very unlikely to be related to the change in drug policy. Nor is it likely that any substantive change in coverage of bed nets could have contributed to the observed difference in oocyst rates because net ownership and use remained relatively low and stable in Rufiji District until late 2005. There were no major changes in availability of nets in Kilombero-Ulanga during the study period (Khatib, Killeen et al. 2008).

Although an efficacious ACT with known gametocytocidal properties was deployed and achieved reasonable population level coverage with an estimated 0.6 to 2.2 AS+SP treatments per person per year, the majority of persons receiving treatment with ACT were symptomatic children. Thus, the asymptomatic, chronically infected, semi-immune older children and adults — who likely constituted the bulk of the reservoir of gametocytes (Ross, Killeen et al. 2006) — were relatively untouched by the introduction of ACT for case management. There have even been suggestions of higher infectivity of gametocytes in asymptomatic carriers in comparison to symptomatic cases due to the large quantity of gametocytes in the former group (Gouagna, Ferguson et al. 2004). In areas where the initial level of malaria transmission is relatively low, the ratio of symptomatic to asymptomatic infections is higher, and larger proportionate reductions in transmission may be likely following introduction of ACTs (Killeen, Ross et al. 2006; Okell, Drakeley et al. 2008; White 2008). Conversely, in areas of high transmission such as investigated here, ACTs may have little impact on prevalence, human population infectiousness and consequent mosquito-to-human transmission because a greater proportion of infections are only mildly symptomatic. Furthermore, even in settings such as these where

artemisinins are combined with complementary partner drugs, such as SP which have long-lasting prophylactic effects (Okell, Drakeley et al. 2008), ACT use may have little impact on overall transmission where it occurs at high intensities simply because individuals often become re-infected within weeks of treatment (Jeffery and Eyles 1955).

ACT might only have a substantial effect on the infectious reservoir if most of the infections are actually being treated with this drug class. The delivery of ACT through public sector outlets in Rufiji rose steadily from 2003 to 2005 with a total of 450,000 doses being deployed for distribution to all registered health facilities by that time (Njau, Goodman et al. 2008), corresponding to a mean consumption rate of 2.22 doses per person per year. Adherence among recipients has been estimated at 75% (Kachur, Khatib et al. 2004), which implies that this drug was delivered reasonably effectively. The proportion of care-seeking visits made to the health facilities that were fever-related rose from 31.8% in 2001 to 54.7% in 2004 (Kachur, Schulden et al. 2006), perhaps due to improved community perceptions, availability and affordability. Recent calls for accurately targeting ACT only to those with patent parasitaemia (WHO 2010) may, paradoxically, further undercut the potential for case management alone to contribute to transmission reduction in highly endemic settings.

While much emphasis has been placed upon the importance of the gametocytocidal properties of ACT, their most important contribution to lowering human population infectiousness is to terminate otherwise long-lasting infections with asexual stages, which intermittently but persistently generate gametocytes and can infect mosquitoes for over a year (Okell, Drakeley et al. 2008). This is comparable to the effect of non-gametocytocidal blood schizonticides. Similarly, the impact of curative drugs upon onward transmission is probably primarily determined by the length of time successfully treated patients remain uninfected — and consequently non-infectious, rather than whether that drug kills the relatively short-lived gametocytes already present at the time of administration. Therefore, while an effective cure may reduce human population infectiousness in an area with little transmission, in parts of Africa where it is common to become re-infected within weeks or even days, even regular treatment of symptomatic infections (Molineaux 1985; Mugittu, Genton et al. 2006) will likely

have only a modest effect upon the proportion of people's lives spent infected and, therefore, on the mean infection prevalence as described (Khatib, Skarbinski et al. 2012).

5. Conclusions

Whilst it is disappointing that no obvious reduction of human infectiousness was evident after introduction of ACT for malaria case management in this first large-area trial in a region of intense transmission, perhaps this is not entirely surprising. Both rapid re-infection and semi-immune, chronically infectious, asymptomatic carriers are common in such settings. The lack of any such secondary benefits in high transmission areas should not detract from the direct public health value of ACT as a means to treat uncomplicated malaria and prevent severe disease manifestations. As has already been outlined in both theory (Okell, Drakeley et al. 2008) and practice (Bhattarai, Ali et al. 2007; Barnes, Chanda et al. 2009), effective chemotherapy with ACT has a vital role in reducing malaria morbidity and mortality. The contribution of chemotherapy to the control and elimination of transmission is likely to be most valuable in settings where transmission is either naturally low or where other approaches such as effective vector control have brought it down to more tractable levels.

There is a need for entomological surveys in parallel to clinical surveillance as a routine component of large-scale trials of anti-malarial drugs or vaccines, but variations in space and time in entomological data should not ignored. Malaria parasite prevalence in vector populations may serve as a useful indicator of the population-wide effect of deployment of interventions that may have only previously been evaluated in individual participants in clinical trials. There is also a need for more cost-effective technologies and procedures for sampling vector mosquito populations across large areas (Kelly-Hope and McKenzie 2009; Sikulu, Govella et al. 2009) to enable accurate and precise measurement of their infection prevalence.

Finally, although there was no demonstrable impact of introducing ACT free for routine case management without diagnostic confirmation, this should not discourage malaria control programmes and their development partners from rolling out interventions to enhance ACT coverage and improve targeting through existing diagnostic tests. Since the study was conceived, ACT and effective vector control through insecticide-treated bed nets have been

scaled up broadly, coinciding with substantial reductions in malaria-related and all-cause child mortality in areas of highly endemic malaria transmission (Roll Back Malaria Partnership 2011). These findings suggest that untargeted ACT alone may have limited impact on transmission. Endemic countries and their development partners should continue to promote ACT and confirmed diagnosis, but may wish to reconsider their expectations of what effect this may have on malaria transmission. Scaling-up and sustaining effective case management along with proven vector control interventions remains the priority for these areas.

Abbreviations

ACT Artemisinin Combination Therapy, CDC Centers for Disease Control, CSP Circumsporozoite Protein, EIR Entomological Inoculation Rate, ELISA Enzyme-Linked Immunosorbent Assay, IMPACT The Interdisciplinary Monitoring Project for Antimalarial Combination Therapy, MTIMBA Malaria Transmission Intensity and Mortality Burden Across Africa, NIMR National Institute of Medical Research and SP Sulphadoxine Pyrimethamine

Competing interests

The authors declare that they have no competing interests. The findings and conclusions in this report are those of the authors and do not represent the official position of the Centers for Disease Control and Prevention.

Authors' contributions

SMKA, TAS, SPK conceived and designed the study. JK and AK led the field data collection. BJH, GFK, TAS, and SMKA analysed and interpreted the data. BJH, GFK, HMF, TAS, SMKA, CL, SPK and JDC drafted the manuscript. GFK, AT, JK and AK provided administrative, technical, and material support. All authors read and approved the final manuscript.

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(Source: http://www.anglicanjournal.com/articles/world-malaria-day-marks-progress-on-fight-to-end-malaria-9732)

Consistently high estimates for the proportion of human exposure to malaria vector populations occurring indoors in rural Africa

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3. Consistently high estimates for the proportion of human exposure to malaria vector populations occurring indoors in rural Africa

3.1 Abstract

Background

Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) are highly effective tools for controlling malaria transmission in Africa because the most important vectors, from the *Anopheles gambiae* complex and the *An. funestus* group, usually prefer biting humans indoors at night.

Methods

Matched surveys of mosquito and human behaviour from six rural sites in Burkina Faso, Tanzania, Zambia, and Kenya, with ITN use ranging from 0.2% to 82.5%, were used to calculate the proportion of human exposure to *Anopheles gambiae* sensu lato and *An. funestus* s.l. that occurs indoors (π_i) as an indicator of the upper limit for the personal protection that indoor vector control measures can provide. This quantity was also estimated through use of a simplified binary analysis (π_i^B) so that the proportions of mosquitoes caught indoors (P_i), and between the first and last hours at which most people are indoors (P_{fl}) could also be calculated as underlying indicators of feeding by mosquitoes indoors or at night, respectively.

Results

The vast majority of human exposure to *Anopheles* bites occurred indoors (π_i^B = 0.79 – 0.97). Neither *An. gambiae* s.l. nor *An. funestus* s.l. strongly preferred feeding indoors (P_i = 0.40 – 0.63 and 0.22 – 0.69, respectively) but they overwhelmingly preferred feeding at times when most humans were indoors (P_{fl} = 0.78 – 1.00 and 0.86 – 1.00, respectively).

Conclusions

These quantitative summaries of behavioural interactions between humans and mosquitoes constitute a remarkably consistent benchmark with which future observations of vector behaviour can be compared. Longitudinal monitoring of these quantities is vital to evaluate the effectiveness of ITNs and IRS and the need for complementary measures that target vectors outdoors.

3.2. Introduction

Insecticide treated nets (ITNs) and indoor residual spraying (IRS) are recognized as the most cost-effective methods for preventing malaria transmission caused by indoor-biting mosquitoes (Lengeler 2004; Pluess, Tanser et al. 2010). The success of these interventions relies on their ability to repel and/or kill endophagic (indoor feeding) mosquitoes, thus providing direct personal protection against exposure to bites, as well as reducing adult mosquito survival, and human-feeding frequency (Lindsay, Adiamah et al. 1991; Magesa, Wilkes et al. 1991; Robert and Carnevale 1991; Lindsay, Adiamah et al. 1992; Howard, Omumbo et al. 2000; Hii, Smith et al. 2001; Maxwell, Msuya et al. 2002; Pates and Curtis 2005). The major malaria vectors of sub-Saharan Africa are *Anopheles gambiae* Giles and *An. arabiensis* Patton from the *Anopheles gambiae* sensu lato species complex and *An. funestus* Giles from the *An. funestu*s s.l. species group (Gillies and DeMeillon 1968). These highly efficient vector species are generally considered to predominantly prefer feeding indoors (endophagic) at night (nocturnal), with peak biting activity typically occurring between midnight and the early hours of the morning when most people are asleep indoors.(Gillies 1988).

However, high coverage rates of ITNs or IRS can dramatically alter vector population composition (Gillies and Smith 1960; Gillies 1962; Gillies and Furlong 1964; Bayoh, Mathias et al. 2010; Russell, Lwetoijera et al. 2010; Bugoro, Cooper et al. 2011; Reddy, Overgaard et al. 2011; Russell, Govella et al. 2011). Consequently, biting activity of the persisting residual populations tends to be more evenly distributed across the night because mosquitoes feeding indoors in the middle of the night are selectively suppressed (Bugoro, Cooper et al. 2011; Reddy, Overgaard et al. 2011; Russell, Govella et al. 2011). Together with emerging resistance to pyrethroids (Ranson, N'Guessan et al. 2011). The only class of existing insecticides suitable for use on ITNs, the host-seeking patterns of such residual vector populations define the limit of how much malaria control can be attained with ITNs and/or IRS, as well as the ideal

properties of complementary vector measures (Griffin, Hollingsworth et al.; Eckhoff 2011; Killeen, Chitnis et al. 2011; Killeen and Moore 2012).

With so few reports with which to compare contemporary observations of changing vector behaviour (Bugoro, Cooper et al. 2011; Reddy, Overgaard et al. 2011; Russell, Govella et al. 2011) and those that may occur in the near future, it is important to examine existing data to get a broader appreciation of the range of values for the proportion of human exposure which has occurred indoors in sub-Saharan Africa. Conventional indices of behavioural patterns of malaria vectors can substantively underestimate the potential protection of ITNs against exposure because they do not consider human indoor and outdoor movements (Govella, Okumu et al. 2010). Here, records of indoor and outdoor mosquito biting distributions from 10 *Anopheles* populations from six rural sites in Africa have been combined with surveys of when humans enter and leave their houses each night in order to understand how much can reasonably be expected from the ongoing scale up of ITNs and IRS (Govella, Okumu et al. 2010; Griffin, Hollingsworth et al. 2010; Eckhoff 2011; Killeen, Chitnis et al. 2011; Killeen and Moore 2012).

3.3 Methods

3.3.1 Study sites

Data were obtained from two multi-country studies spanning six rural sites in southern, eastern and central Africa (Figure 1, Table 1). Included in this analysis are two sites in Burkina Faso (Oubritenga and Kourweogo) and two in Tanzania (Ulanga and Rufiji) that were in the Malaria Transmission Intensity and Mortality Burden Across Africa (MTIMBA) study carried out between 2001 and 2004, together with one site in Zambia (Luangwa) and one in Kenya (Rarieda) that were in the Malaria Transmission Consortium (MTC) study, carried out between 2009 and 2010. The two sites in Burkina Faso had little coverage with any vector control measure at the time of the MTIMBA study, the two Tanzanian sites had low coverage with nets, and the sites in Zambia and Kenya had high coverage with ITNs. None of the sites were

covered by an IRS programme. Note, however, that in the case of the Kenyan site, incremental impact of IRS upon malaria transmission was observed nearby when Rachuonyo district was sprayed with the synthetic pyrethroid lambda-cyhalothrin (Hamel, Otieno et al. 2011). None of the houses in which HLC was conducted had been sprayed with any residual insecticides.

Figure 4 Map of Africa showing locations of study sites.

3.3.2 Mosquito behaviour surveys

Mosquito biting rates were observed hourly during the night both indoors and outdoors, by human landing catch (HLC) method (Service 1977), with collectors using an aspirator and torchlight to catch mosquitoes landing on their exposed legs. At the MTIMBA study sites, except for Ulanga, two collectors interchanged their positions between indoors and outdoors every hour, and the pair was replaced by a second pair of collectors after the sixth hour. In the MTC study sites and the Ulanga site, a pair of collectors (one collector stationed indoors and one outdoors) did the collection throughout the night for 45 minutes every hour with a 15 minutes break (Killeen, Kihonda et al. 2006). The HLC exercise began at 18.00 hours in Rarieda and Rufiji, 19.00 hours in Lupiro and Luangwa, and at 20.00 in Oubritenga and Kourweogo. HLC surveys finished at 06.00 hours in Rarieda and Rufiji and 07.00 hours in all the other sites.

3.3.3 Human behaviour surveys

Surveys of human behaviour were used to determine which hours residents spent indoors and outdoors at night. The MTIMBA and MTC studies used different methods for this. In the MTIMBA study, direct observations were recorded by a field worker who sat in a randomly selected compound and recorded the number of people that were awake at hourly intervals from 6.00pm until they all retired indoors. A similar procedure was carried out on the same compound from 4.00 am to 6.00am on the following morning. In the MTC study, four questions were incorporated into standard cross-sectional malaria indicator survey questionnaires asking when, to the nearest hour, the respondent went indoors for the night, went to bed to sleep, awoke in the morning, and left the house in the morning.

Table 3 Description of study sites [a]

Site	Geographical coordinates	Duration	Study*	Intervention(s)	ITN Use[b]**	Dominant vector species	
						An. gambiae s.l.	*An. funestus* s.l.
Kenya Rarieda	0. 18 S 34.40 E	2009	MTC	ITNs & IRS	82.5%	*An. arabiensis*	*An. funestus* s.s.
Zambia Luangwa	15.13 S 30.20 E	2009-10	MTC	ITNs & IRS	66.0%	*An. quadrianulatus*	*An. funestus* s.s.
Tanzania Rufiji	7.95 S 38.98 E	2002-04	MTIMBA	ITNs	25.4%	*An. gambiae* s.s.	*An. funestus* s.s.
Tanzania Ulanga	8.35 S 36.67 E	2002-04	MTIMBA	ITNs	<20%	*An. gambiae* s.s.	*An. funestus* s.s.
Burkina Faso Oubritenga	12.73 N 1.44 W	2002-04	MTIMBA	ITCs & ITNs	0.6%	*An. gambiae* s.s.	*An. funestus* s.s.
Burkina Faso Kourweogo	12.73 N 1.75 W	2002-04	MTIMBA	ITNs	0.2%	*An. gambiae* s.s.	*An. funestus* s.s.

[a]ITN = insecticide treated net; ITC = insecticide treated curtains; MTC = Malaria Transmission Consortium;

MTIMBA = Malaria Transmission Intensity and Mortality Burden Across Africa

[b]Proportion of children < 5 years old who reported using an ITN during the night before the survey.

3.3.4 Data analysis

Several studies calculate the average proportion of human exposure to bites of a given vector population which occurs indoors in the absence of any protective measure such as an ITN (π_i) (Govella, Okumu et al. 2010; Bugoro, Cooper et al. 2011; Russell, Govella et al. 2011; Seyoum, Sikaala et al. 2012). This parameter limits the possible degree of any exclusively indoor measure can provide, and therefore the consequent level of indirect protection achieved through community-wide suppression of mosquito longevity, feeding frequency and access to humans (Killeen, Chitnis et al. 2011; Killeen and Moore 2012; Kiware, Chitnis et al. 2012). This epidemiologically critical upper limit for personal protection and key determinant of community-level protection (Kiware, Chitnis et al. 2012) was initially calculated (Figure 3) by weighting the mean indoor and outdoor biting rates for each hour of the night by the proportion of humans reporting to have been indoors and outdoors, respectively, at that time (Seyoum, Sikaala et al. 2012). In order to facilitate a consistent mathematical description of this calculation, a sequence of 24 hour-long intervals is defined that begins at 18.00 hours on the conventional 24 hour clock so that $t = 0$ corresponds to the period from 18.00 to 19.00 hours, $t = 1$ corresponds to 19.00 to 20.00 hours, continuing through to $t = 23$ for the period from 17.00 to 18.00 hours (Seyoum, Sikaala et al. 2012). The proportion of human exposure to bites by a given vector population which occurs when residents are both indoors and sleeping or trying to sleep (π_s) was calculated similarly to π_i, using the same denominator estimate of total indoor and outdoor exposure, but a numerator which is the sum of the products of the mean indoor biting rates and the estimated proportions of humans reporting to have gone to bed to sleep for each hour of the night (Seyoum, Sikaala et al. 2012).

The proportion of exposure to mosquito bites of unprotected individuals which occurs indoors (π_i^B) was also estimated in a more simplified binomial fashion, so that it could be analyzed by logistic regression (Bugoro, Cooper et al. 2011; Russell, Govella et al. 2011; Seyoum, Sikaala et al. 2012) using generalized linear models (GLMs) specifically designed to quantify the influence

of categorical or continuous independent variables upon binary dependent variables (Table 2)(Collett 2002). The nightly interval that is considered as normally spent indoors was defined as beginning at the first (f) and ending at the last (l) hour when the majority of people were indoors so that π_i^B could be calculated simply as the total number of mosquitoes caught indoors during that period, divided by the sum of this total and the total caught outdoors before and after this interval (Seyoum, Sikaala et al. 2012).

In order to more clearly interpret the estimates obtained, two underlying determinants of π_i^B were also calculated exactly as described recently (Seyoum, Sikaala et al. 2012). These were: (i) the propensity of vectors to feed indoors is reflected in the proportion of all mosquitoes caught that were captured indoors (P_i); and (ii) the propensity of vectors to feed at times when people are indoors, which is reflected in the proportion of all mosquitoes caught that were captured during hours when the majority of people were indoors (P_{fl}). These crude binomial estimates of P_i, P_{fl} and π_i^B allowed statistical comparisons through logistic regression, using generalized linear models (GLM) with a logit link function and binomial distribution (Collett 2002) for these binary outcomes (PASW Statistics, version 18). Comparisons were made across categorical explanatory variables of site and species for tendency towards endophagy and nocturnal activity. Vector preference for both feeding indoors (P_i) and at times when most humans were indoors (P_{fl}) was compared with the null hypotheses (P_i or $P_{fl} = 0.5$). The first (f) and last (l) hour during which most of the human population was indoors were estimated separately for each site based on the surveys of human behaviour described above.

3.3.5 Protection of human subjects and ethical approval

Ethical clearance was obtained from local ethical review bodies. Humans participating in the HLC exercise were made aware of the study procedures and risks involved by their participation. Necessary precautions were taken such as regular screening for malaria parasites

and prompt treatment of positive cases based on the prevailing malaria treatment guidelines. In Rarieda and Luangwa, collectors were provided with the malaria prophylaxis Lariam® (Mefloquine) and Malarone® (Atovaquone-Proguanil Hydrochoride), respectively. Prior to visiting households for human behaviour surveys, permission was sought from the appropriate local authorities.

3.4 Results

Vector behavioural patterns differed considerably between locations and taxa, with peaks of biting activity occurring anytime from just after dusk to just before dawn (Figure 2). Biting rates that were obviously higher indoors than outdoors were not as ubiquitous as expected and occurred in only four of the *An. gambiae* s.l. populations and one of the *An. funestus* s.l. populations. Figure 2 illustrates a substantial degree of diversity in human and mosquito behaviour across Africa. The amount of time that residents spent indoors during the night varied from 8 hours in Ulanga to 12 hours in Rarieda.

Despite all the diversity manifested in the 10 vector populations and the six human populations illustrated in Figure 2, Figure 3 depicts a remarkably consistent picture in terms of the generally high proportions of human exposure to mosquito bites that occur indoors. Although no data describing when residents slept (rather than merely spent indoors), were available for most of these sites, these data were available for both Rarieda and Luangwa for which the more directly relevant proportion of exposure occurring while indoors and asleep (π_s) was calculated. In Rarieda, where remarkably endophilic human behaviour would raise the greatest concern that the proportion of exposure occurring indoors (π_i) would overestimate the true fraction of exposure directly preventable by an ITN while sleeping (π_s), there were modest differences between the estimates of π_i and π_s, with the latter estimated as 0.82 for *An. gambiae* s.l. and 0.92 for *An. funestus* s.l., whereas the former was estimated to

be 0.95 and 0.97, respectively (Figure 3). Similarly, the proportions of human exposure to mosquito bites occurring while asleep in Luangwa were also high, being 0.77 for *An. gambiae* s.l. and 0.86 for *An. funestus* s.l., compared to π_i values of 0.89 and 0.92, respectively.

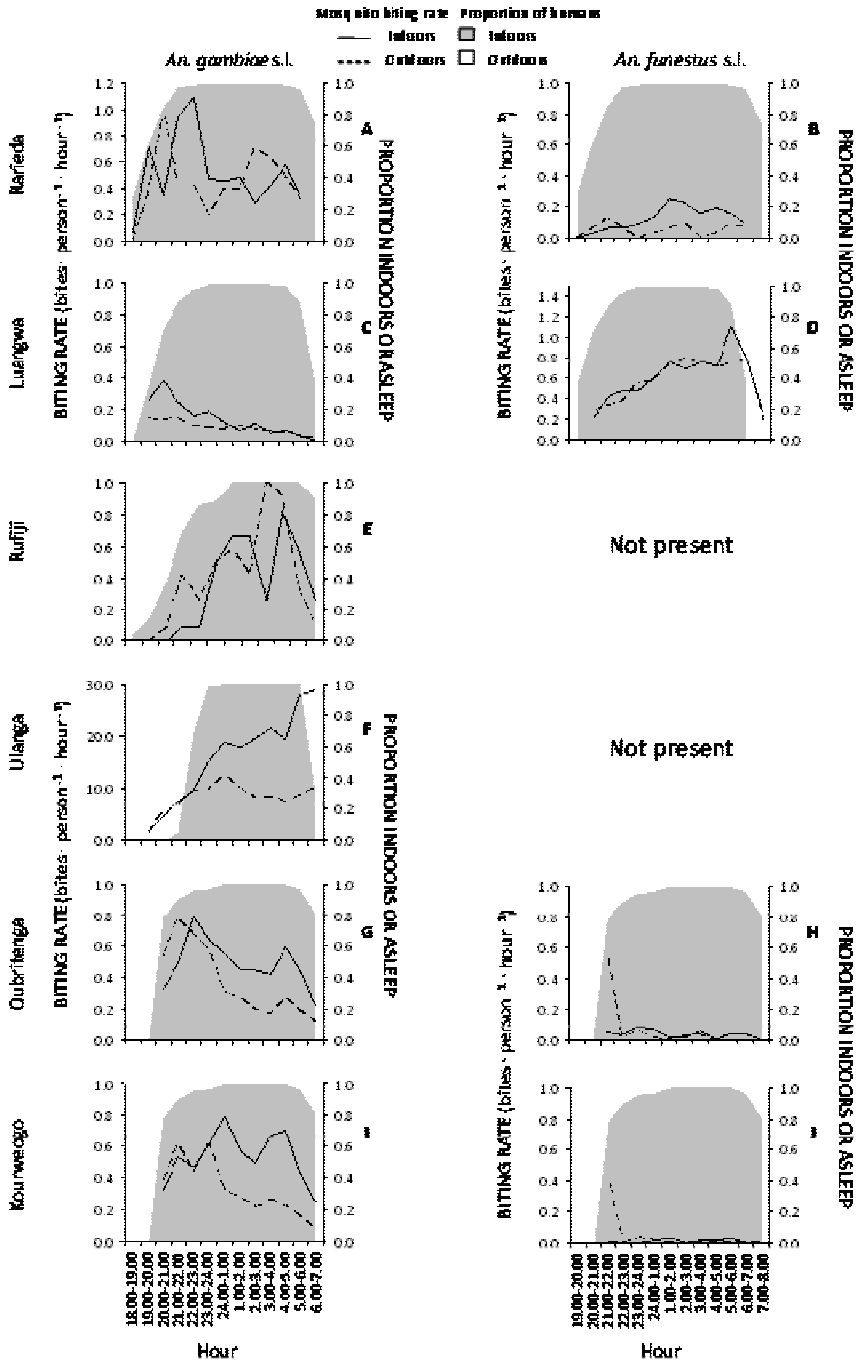

Figure 2. Hourly biting pattern of *Anopheles gambiae* sensu lato (panels on the left) and *Anopheles funestus* sensu lato (panels on the right) occurring both indoors (solid line) and outdoors (dashed line) in the different study sites. The grey area represents the proportion of the human population predominantly spending time indoors during the times shown on the abscissa of each graph.

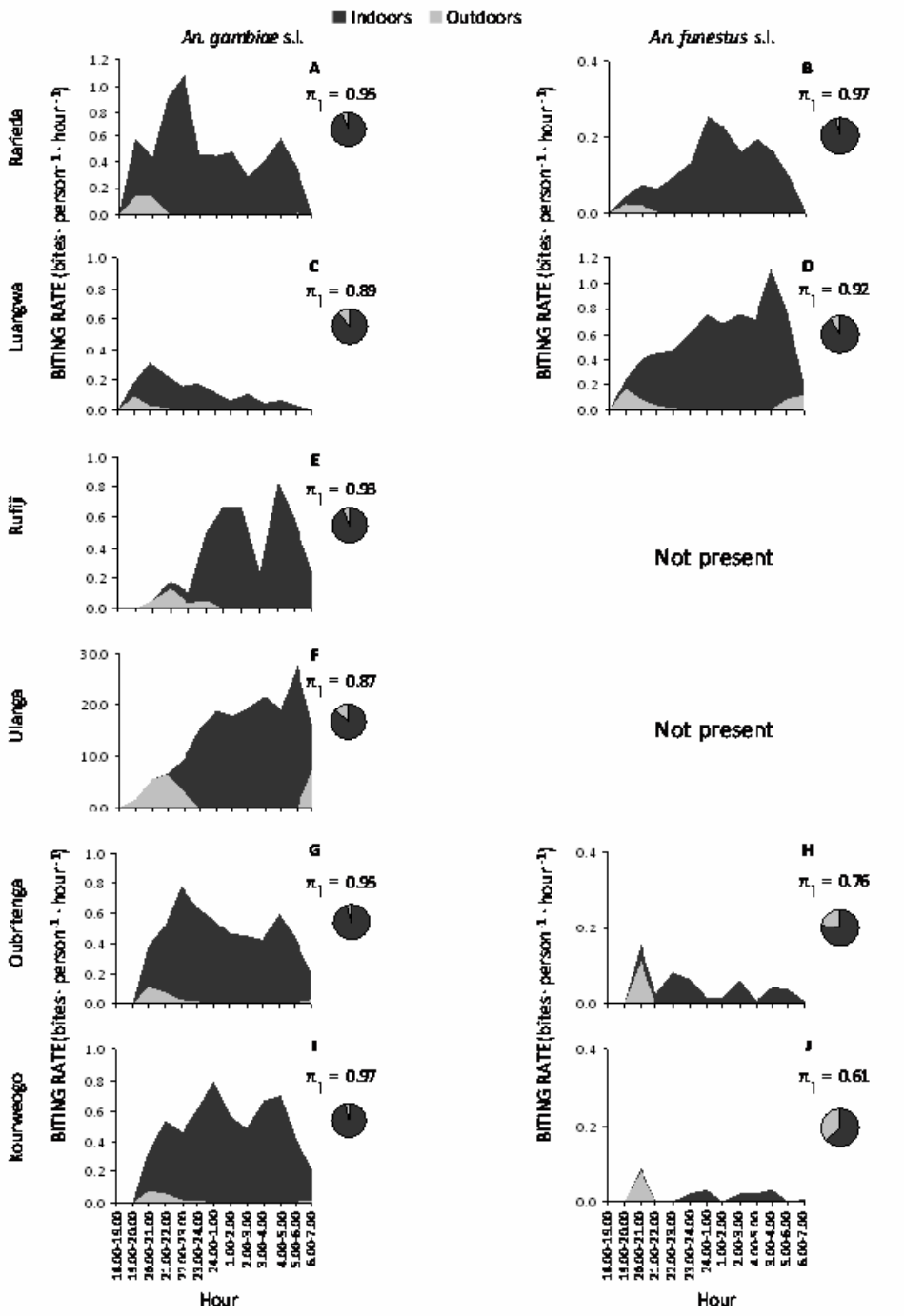

Figure 3 Exposure profiles of the experienced by the human population both *Anopheles. gambiae* sensu lato (panels on the left) and *Anopheles funestus* sensu lato *(*panels on the right) experienced by human population at different study sites. Pie charts illustrate the amount of exposure occurring indoors and outdoors. The light grey areas in the graphs and pie charts represent outdoor human exposure and the dark grey area represents indoor human exposure.

Examining the binomial estimates of the proportion of human exposure occurring indoors (π_i^B) in the context of its two explanatory quantities (P_i and P_{fl}) cleary shows even greater consistency across all these Anopheline-human population interactions (Table 2). Consistent with estimates obtained by weighting indoor and outdoor vector biting rates according to the proportion of humans n those categories (Figure 3), the simple binomial estimates described in Table 2 indicate that almost all human exposure to members of the *An. gambiae* complex and the *An. funestus* group occurred indoors (π_i^B,= 0.79-1.00 and 0.88-1.00, respectively). Interestingly, mosquito preferences for feeding indoors did not appear to be a strong driver of this epidemiologically crucial quantity (Govella, Okumu et al. 2010; Griffin, Hollingsworth et al. 2010; Eckhoff 2011; Killeen, Chitnis et al. 2011; Killeen and Moore 2012) with P_i ranging from 0.40 to 0.63 for *An. gambiae* s.l. and from 0.22 to 0.69 for *An. funestus* s.l. Although *An. gambiae* s.l. populations in Luangwa, Korouwego and Oubritenga, and *An. funestus* s.l. populations in Rarieda and Luangwa exhibited a clear preference for feeding indoors ($P_i > 0.5$), the magnitude of these preferences were modest and cannot explain the high values for π_i in these sites. Furthermore, human exposure to *An. funestus* s.l. at both the Kourowego and Oubritenga sites in Burkina Faso occurred mostly indoors ($\pi_i \geq 0.61$) despite the apparent preference of these vector populations for feeding outdoors ($P_i < 0.5$).

In stark contrast, estimates for the proportion of mosquitoes caught between the first and last hour when most humans were indoors (P_{fl}) were consistently high, ranging from 0.78 to 1.00 for *An. gambiae* s.l. and from 0.86 to 1.00 for *An. funestus* s.l. (Table 2). In the absence of any evidence for strong mosquito preference for feeding indoors in the strict sense ($P_i >> 0.5$), it appears to be the ubiquitously strong preference for feeding at times of the night when most humans are indoors ($P_{fl} \geq 0.78$) that primarily drives the consistently high proportion of human exposure that occurs indoors across Africa.

Table 2. Estimates of the proportion of mosquitoes caught indoors (P_i), the proportion of mosquitoes caught between the first and last hour when most humans were indoors (P_{fl}), the proportion of human exposure to mosquito bites occurring indoors, weighted by human behaviour (π_i), or calculated in a binomial fashion (π_i^B), for *Anopheles gambiae* sensu lato and *An. funestus* s.l. in six sites in Africa.

	n[a]	P_i [95% CI]	*P*-value	P_{fl} [95% CI]	*P*-value	n[b]	π_i^B [95% CI]	*P*-value
An. gambiae s.l.		Overall effect of site:	<0.001	Overall effect of site:	<0.001		Overall effect of site:	<0.001
		χ2=66.80, df=5		χ2=29.49, df=3			χ^2=18.20, df=3	
Rarieda	337	0.54 [0.48,0.59]	0.174*	0.78 [0.73,0.82]	<0.001*	187	0.79 [0.72,0.84]	<0.001*
Luangwa	638	0.63 [0.60,0.67]	<0.001*	0.84 [0.81,0.87]	<0.001*	380	0.90 [0.87,0.93]	<0.001*
Rufiji	102	0.46 [0.36,0.56]	0.429*	0.99 [0.93,1.00]	<0.001*	48	0.98 [0.87,1.00]	<0.001*
Ulanga	320	0.40 [0.34,0.45]	<0.001*	0.91 [0.88,0.94]	<0.001*	127	0.89[0.82,0.93]	<0.001*
Oubritenga	1377	0.57 [0.55,0.60]	<0.001*	1.00**		791	1.00**	
Kourweogo	1019	0.62 [0.59,0.65]	<0.001*	1.00**		637	1.00**	
An. funestus s.l.		Overall effect of site:	<0.001	Overall effect of site:	0.023		Overall effect of site:	0.08
		χ2=44.19, df=4		χ2=5.18, df=1			χ2=2.91, df=1	
Rarieda	71	0.69 [0.57,0.79]	0.003*	0.86 [0.75,0.93]	<0.001*	48	0.88 [0.75,0.94]	<0.001*
Luangwa	3384	0.52 [0.50,0.53]	0.050*	0.93 [0.93,0.94]	<0.001*	1746	0.94 [0.93,0.95]	<0.001*
Oubritenga	155	0.36 [0.29,0.44]	<0.001*	1.00**		56	1.00**	
Kourweogo	62	0.22 [0.14,0.34]	<0.001*	1.00**		14	1.00**	

n = number of mosquitoes included in each analysis. P-value = estimated probability of the null hypothesis for each analysis. Df, degrees of freedom.

[a]Total number of mosquitoes caught. [b]Total number of mosquitoes sampled in the evening outdoors, at night indoors and in the morning outdoors.

[c]Estimated probability for the null hypothesis of a value equal to 0.5. [d]Confidence interval could not be determined, as al mosquitoes were in one category.

3.5 Discussion

Apart from a scope that spans only six sites in four countries, this study has a number of limitations relating to the technical methodology applied. Previous comparisons of questionnaires with direct observations for surveying the human behaviours suggest that these are approximately but not entirely consistent with each other (Geissbühler, Chaki et al. 2007). In terms of mosquito behaviour, several of the sites may not have captured some low levels of outdoor human exposure that occurred before human landing catch surveys started in the evening and after they ended in the morning. The proportion of human exposure occurring indoors (π_i) may therefore have been slightly overestimated. However, examining the trends on either end of the activity profiles in figure 3 reveals that in no case is this likely to result in overestimation by more than 10%. Additionally, the accuracy of these mosquito surveys is limited to some extent by the practical challenge of maintaining consistently sensitive human landing catches throughout the night (Service 1977). Lack of explicit molecular data to distinguish sibling species and molecular forms within the major taxa occurring in both Tanzania and Burkina Faso also introduces ambiguity to the interpretation of the results. This limitation if of greatest significance for the Burkina Faso sites where both populations of *An. funestus* s.l. exhibited early peaks of outdoor biting activity (Figure 2H and J) that contrast clearly with historical observations of feeding activity peaks that occurred indoors during sleeping hours for both *An. gambiae* sensu stricto and *An. funestus* sensu stricto in other areas of Burkina Faso (Robert, Carnevale et al. 1988; Robert and Carnevale 1991). In the absence of molecular data with which to distinguish exactly which members of the *An. funestus* s.l. group contributed to these observations, we can only conclude that these distinct, early peaks of outdoor exposure may well be accounted for by secondary vectors, such as *An. rivulorum* or *An. parensis* (Gillies and DeMeillon 1968; Gillies and Coetzee 1987; Ilboudo-Sanogo, Cuzin-Ouattara et al. 2001; Dabire, Baldet et al. 2007), that can replace *An. funestus* sensu stricto when selective pressure is applied by vector control but are of negligible relevance to malaria transmission (Gillies and Smith 1960; Matola, ljumba et al. 1990; Magesa, Wilkes et al. 1991).This phenomenon also explain the discrepancy between the behaviour weighted (Figure

3) and simpler binomial estimates (π_i^B, Table 2), and suggests that the latter may be more representative of exposure to important primary vectors: The more subtle weighted estimate captures the brief but intense period of largely outdoor exposure of a minority of residents to these peaks of biting activity by presumably secondary vectors that occur between 20:00 and 21:00 hours (Figure 2).

However, the most important limitations of this study are fundamental in nature and relate to the relevance of the π_i parameter itself. Estimates of the proportion of mosquitoes which make contact with treatable surfaces while resting within houses (π_r) would be far more directly relevant to community-level transmission control with IRS rather than ITNs but field methods for measuring such a quantity have yet to be developed. Also, the proportion of exposure occurring while asleep (π_s) is a more directly relevant determinant of protection with ITNs than π_i but could only be estimated for the Kenyan and Zambian sites. The assumption that the latter only modestly overestimates the former obviously introduces some degree of systematic inaccuracy. Examining the two sites for which both quantities are estimable suggests quite modest differences between these alternative estimates of individual protective coverage. However, a very different picture emerges when the same estimates are considered in terms of the protective coverage gaps that allow malaria transmission and vectors populations to persist, highlighting the crucial importance of high biological coverage and accurate ways to measure it (Kiware, Chitnis et al. 2012). In Rarieda, biological coverage gaps of only 5% for *An. gambiae* s.l. and 2% for *An. funestus* s.l. are apparent when calculated as the complement of π_i, but this contrasts dramatically with values of 18% and 8%, respectively for the complement of π_s. In Luangwa, corresponding coverage gaps of 11% and 8% when estimated based on π_i are approximately doubled to 23% and 14% when based on the π_s measurement, which more accurately reflects protective coverage with nets.

Despite these limitations, a number of clear, useful and broadly applicable conclusions can nevertheless be drawn. It appears that the mosquito taxa which are responsible for most of the malaria transmission in Africa have only mild and inconsistent preferences for feeding

indoors. However, biting contact with humans overwhelmingly occurs indoors simply because this is where people spend the most of the hours of darkness when these vectors are active. These findings are consistent with the long-standing rationale for prioritization of ITNs and IRS for malaria prevention in Africa and support their continued upscale across the continent (World Health 2007; Flaxman, Fullman et al. 2010). However, some human exposure to vector mosquitoes occurred outdoors in all sites (Figure 3) so additional vector control measures (Ferguson, Dornhaus et al. 2010) that complement ITNs and IRS by targeting this gap in *de facto* protective coverage may well be required if malaria transmission is to be eliminated in such settings (Killeen and Moore 2012).

It is particularly encouraging that most of the human-vector interaction occurred indoors in the most recently surveyed Rarieda and Luangwa sites which both had high ITN coverage at that time. In the Rarieda site, ITNs have had a clear (Hawley, ter Kuile et al. 2003; Phillips-Howard, Nahlen et al. 2003) and sustained (Lindblade, Eisele et al. 2004; Eisele 2005; Shah, Kariuki et al. 2011) impact upon malaria transmission, morbidity and mortality. Furthermore, substantive changes in vector population composition have occurred with *An. gambiae* s.s. all but disappearing, leaving *An. arabiensis,* which is known to be capable of feeding extensively on humans early in the evenings before humans go indoors,(Govella, Okumu et al. 2010; Russell, Lwetoijera et al. 2010; Yohanne and Boelee 2012) as the only remaining vector species from the *An. gambiae* s.l. *c*omplex (Bayoh, Mathias et al. 2010). The continued high proportions of human exposure to transmission occurring indoors in the absence of personal use of an ITN (π_i) up to at least 2009 may well help explain why supplementing ITNs with IRS confers additional incremental protection in a nearby district,(Hamel, Otieno et al. 2011) despite dramatic changes in vector population composition, and may underpin similar observations elsewhere (Kleinschmidt, Schwabe et al. 2009).

However, these continued high proportions of human exposure to bites by extensively modified residual vector populations (Bayoh, Mathias et al. 2010) in Rarieda contrast strongly with recent observations of dramatic declines in this proportion following ITN and IRS scale up in Equatorial Guinea (Reddy, Overgaard et al. 2011) and in the Ulanga site itself (Russell,

Govella et al. 2011), as well outside of Africa in the Solomon Islands (Bugoro, Cooper et al. 2011). It is therefore clear that summary estimates of relevant mosquito-human interaction quantities, such as P_i, P_{fl} and π_i should be regularly monitored by national malaria control programmes and carefully considered by policy makers, product manufacturers and public health funding bodies (Eckhoff 2011; Killeen, Chitnis et al. 2011; Killeen and Moore 2012). Care should be taken not to misinterpret such reports of declining proportions of human exposure occurring indoors: These measurements do not necessarily reflect a failure of ITNs or IRS. Instead, these often represent the characteristics of persisting populations of zoophagic and exophagic mosquitoes following successful control (Gillies and Smith 1960; Matola, ljumba et al. 1990; Magesa, Wilkes et al. 1991; Bayoh, Mathias et al. 2010; Russell, Lwetoijera et al. 2010; Meyrowitsch, Pedersen et al. 2011; Reddy, Overgaard et al. 2011; Russell, Govella et al. 2011) and even elimination (Bugoro, Cooper et al. 2011) of anthropophagic and endophagic vector populations by ITNs or IRS. By definition, less anthropophagic mosquitoes are less efficient vectors of these malaria parasite species because *Plasmodium falciparum* and *P. vivax* are strict anthroponoses that only infect human hosts. Indeed many of these, such as the *An. quadriannulatus, An. rivulorum, An. parensis, An. vaneedeni* and *An. leesoni* found in Luangwa, are considered to play a negligible role in sustaining transmission of malaria (Gillies and DeMeillon 1968; Gillies and Coetzee 1987). It may therefore be inappropriate to judge the ongoing effectiveness of commonly used vector control measures such as ITNs on the basis of contemporary measures of mosquito-human interactions because these reflect the characteristics of the surviving mosquito populations only. Quantitative estimates of behavioural parameters, such as those presented here (Figures 3, Table 2), collected before scale up of ITNs or IRS (Ulanga, Kourowego, Oubritenga), or at least before these interventions had substantially lowered π_i values (Rarieda, Luangwa, Rufiji), may therefore be more representative than contemporary measurements for evaluating the ongoing impact of ITNs on vectors of historical importance. Such historical reference values are therefore crucial to balanced interpretation of contemporary estimates and observation of longitudinal trends. The consistency of the summary values presented in Table 2 suggests it may be reasonable to

extrapolate this range of values beyond these 6 study sites so they may even constitute useful historical reference values for rural African vector populations generally.

Despite the limitations described in the two opening paragraphs of this discussion, measurements of π_i are very useful for approximately assessing *de facto* protective coverage of humans with ITNs and IRS (Killeen, Chitnis et al. 2011; Kiware, Chitnis et al. 2012; Seyoum, Sikaala et al. 2012). The proportion of human exposure to bites which occurs indoors can be most directly applied to estimating the maximum level of personal protection that can be realistically expected with indoor vector control measures, or combinations thereof (Seyoum, Sikaala et al. 2012). However, the relevance of this behavioural parameter extends far beyond personal protection because it is critically important as a determinant of the greater community-level impacts ITNs and IRS can deliver when used by the majority of the population (Killeen, Chitnis et al. 2011). Even though π_i does not directly reflect probability of insecticide contact while resting, the high estimates for Rarieda help rationalize evidence for incremental impact of IRS as a supplement to ITNs in a neighbouring district (Hamel, Otieno et al. 2011). In Luangwa, similar estimates have been used to infer that IRS may also be a useful supplement to ITNs in that setting (Seyoum, Sikaala et al. 2012) and the consistently high values presented here are consistent with recent reviews suggesting this combination may have broad potential in Africa (Kleinschmidt, Schwabe et al. 2009; Okumu and Moore 2011).

Beyond IRS and ITNs, π_i is also informative as a primary determinant of target product profiles for complementary measures designed to fill the coverage gaps created when mosquitoes feed outdoors (Killeen and Moore 2012; Kiware, Chitnis et al. 2012). It has long been recognized that pre-existing behavioural resistance traits, specifically preferences for feeding outdoors, usually limit the impact of vector control far more than physiological resistance to the relevant active ingredients of insecticides (Muirhead-Thomson 1951; Muirhead-Thomson 1960; Elliott 1972). In fact, many of the diverse primary vectors distributed across tropical America and Asia

are predominantly exophagic (Muirhead-Thomson 1951; Muirhead-Thomson 1960; Elliott 1972; Trung, Bortel et al. 2005; Van Bortel, Trung et al. 2010). Furthermore, residual mosquito populations that persist following ITN and IRS scale up in Africa and the Pacific are often perfectly capable of mediating stable, endemic transmission because they include primary vectors that are behaviourally resistant to these measures (Bayoh, Mathias et al. 2010; Govella, Okumu et al. 2010; Bugoro, Cooper et al. 2011; Reddy, Overgaard et al. 2011; Russell, Govella et al. 2011; Trape, Tall et al. 2011; Moiroux, Gomez et al. 2012; Yohanne and Boelee 2012). The primary parameter that determines the comparative merits of vapour phase insecticides which can be used in outdoor spaces, as opposed to contact insecticides, which by definition require a treatable surface to which they can be applied, is the proportion of human exposure occurring indoors (Killeen and Moore 2012; Kiware, Chitnis et al. 2012). The consistently high values for this quantity reported in Figures 3 and Table 2 confirm that ITNs and IRS using contact insecticides are indeed the logical first intervention choice while the intermediate values reported recently from residual populations across the tropics (Govella, Okumu et al. 2010; Bugoro, Cooper et al. 2011; Russell, Govella et al. 2011) suggest that supplementary use of vapour phase repellents may well complement these traditional approaches effectively in such situations (Killeen and Moore 2012). In addition to the usual assays of physiological susceptibility to insecticides that are already integral to choosing vector control measures (WHO 2006; Ranson, N'Guessan et al. 2011) up-to-date surveys of vector behavioural characteristics will also be essential to underpin selection of alternative or additional vector control technologies.

A recent modelling analysis cautions that supplementing existing ITNs or IRS with indoor use of spatial repellents may undermine and reverse impact of the former upon historically important anthropophagic and endophagic vectors that have been suppressed but persist and can therefore recover if they are deterred from houses where they would otherwise be killed (Killeen and Moore 2012). When deciding about whether to supplement ITNs with IRS, it is therefore essential to consider, not only the contemporary values of such behavioural

quantities for surviving residual vector populations, but also the normal range of values for historically important vectors that need to be suppressed indefinitely (Kiware, Chitnis et al. 2012). To conclude, we recommend that historical values for such behavioural parameters recorded before wholesale changes in vector population composition are likely to be more useful for rationalizing the impact of ongoing interventions while equivalent, contemporary surveys of surviving residual populations are more appropriate for informing strategies to augment existing control tools and ultimately eliminate transmission of malaria (Kiware, Chitnis et al. 2012).

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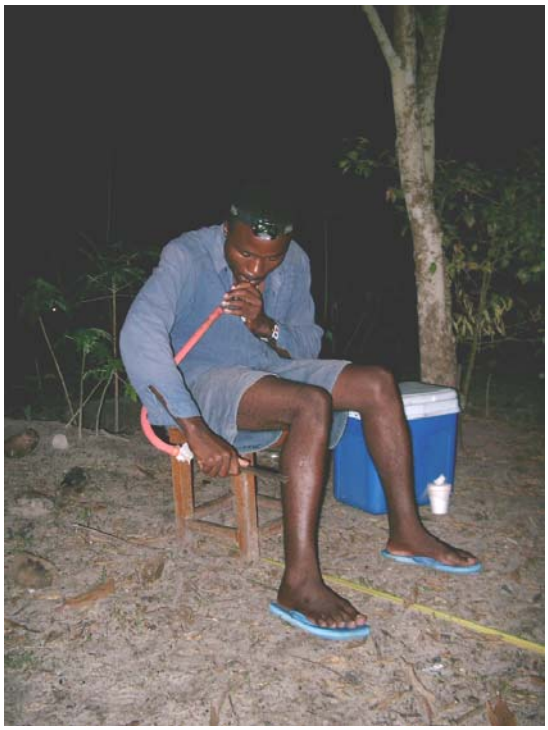
Conflict of interest

The authors declare that they have no conflict of interest.

KEY MESSAGES

- African malaria vectors have no strong or consistent preference for feeding indoors.
- Nevertheless, most human exposure to biting malaria vectors occurs indoors because that is where humans sleep during peak hours of feeding activity.

- Mosquito feeding patterns should be monitored longitudinally to enable rational management of vector control programmes and guide optimal formulation of target product profiles for new control technologies.



Person performing human landings catch (Courtesy of N. Govella)

Inconsistency in the relative performance of human landing catches and light traps in sampling anopheline populations across ecological zones of Africa

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4 Inconsistency in the relative performance of human landing catches and light traps in sampling anopheline populations across ecological zones of Africa

4.1 Abstract

The need for surveillance of adult host seeking mosquitoes is of foremost importance in determining levels of disease transmission and for appropriate allocation of interventions. The gold standard for estimating mosquito – human contact rate has thus far been obtained based on Human Landing Catch (HLC), where human volunteers catch mosquitoes that land on their exposed body parts. This approach necessitates exposure to potentially infectious mosquitoes, such a risk it is unethical calling the need for safer and accurate tools. Centers for disease control light traps (LT) have been used widely in malaria endemic setting as an alternative tool to HLC in estimating human biting rate (HBR). Here, multi sites paired mosquito collections of LT against HLC are evaluated for their consistency in sampling indoor host seeking mosquitoes. Regression models were applied to determine the site specific as well as the overall LT sampling efficiency and their trend across increasing mosquito density for two major malaria vectors across Africa, *Anopheles gambiae* sensu lato and *Anopheles funestus* sensu lato. Generally, LT were able to collect more mosquitoes than HLC, though the ratio of LT:HLC varied between sites and mosquito density. Across sites LT had an overall sampling efficiency of $\tilde{\alpha}_t$ =1.07 [0.76-1.51] in sampling *An. gambiae* s.l. and $\tilde{\alpha}_t$ =1.78 [0.90-3.44] in sampling *An. funestus* s.l.. There was variation in sampling efficiency of LT across mosquito densities and only in a few locations did LT sample proportionally to HLC. More often LT either underestimated human exposure by under-sampling or over-sampling at high mosquito densities, in particular for *An. funestus*. Such inconsistency necessitates calibration of LT against HLC for each location and across seasons. We also advise against the use of a single calibration factor across all geographical locations since no evidence of a geographical pattern in the sampling efficiency of LT against HLC was demonstrated.

4.2 Introduction

Estimation of the rate of contact between mosquitoes and humans is essential in evaluating the extent of human exposure to mosquitoes as well as in projecting possible impacts that exposure reducing interventions might have on lowering transmission intensity. Samples of host-seeking mosquitoes can be used in estimating the human biting rate (HBR), a basic parameter in assessing transmission of any mosquito borne disease (MacDonald 1957). HBR when multiplied with the prevalence of sporozoites in mosquitoes gives an estimate of the entomological inoculation rate (EIR), a direct measure of malaria transmission intensity (Beier, Killeen et al. 1999).

Human landing catch (HLC) is the 'gold standard' method in determining the extent of biting by host-seeking mosquitoes and the extent of exposure of humans to mosquito bites, at a given time and location (WHO 1975; Service 1977). However, this method is ethically questionable due to the deliberate exposure of humans to potentially infectious bites. It is also uncomfortable, and labour intensive and difficult to supervise. Furthermore, individuals vary in their attractiveness to mosquitoes (Brouwer 1960; Knols 1996; Takken and Knols 1999) as well as in their ability to catch mosquitoes (Service 1977). HLC methods may overestimate exposure in areas where different vector control interventions have been widely rolled out, such as bed-nets , repellents , and screened houses , if not corrected for these factors.

Several other methods that do not require human exposure have been tested in an attempt to find an alternative to HLC for estimating the HBR. Light traps placed near an occupied bed net inside a house (Odetoyinbo 1968; Lines, Curtis et al. 1991; Mbogo, Glass et al. 1993; Davis, Hall et al. 1995), Mbita bed net trap (Mathenge, Killeen et al. 2002; Mathenge, Omweri et al. 2004; Mathenge, Misiani et al. 2005), tent traps such as the Ifakara tent trap and Furvella trap (Govella, Chaki et al. 2009; Govella, Chaki et al. 2011) and odour-baited traps (Jawara, Smallegange et al. 2009). Among these methods, the Centers for Disease Control light trap (LT) is the most widely used and broadly accepted method for trapping host seeking mosquitoes (Odetoyinbo 1968; Garrett-Jones and Magayuka 1975; Lines, Curtis et al. 1991; Magbity, Lines et al. 2002).

The sampling efficiency and bias of LT as compared to HLC has been evaluated in several areas with diverse outcomes (Lines, Curtis et al. 1991; Mbogo, Glass et al. 1993; Davis, Hall et al. 1995; Hii, Smith et al. 2000). The differences observed could be due to methodological differences, both in placement of the trap (Mboera, Kihonda et al. 1998) and in data analysis (Smith 1995), or due to spatial and temporal variations in mosquito behaviour. The analysis presented here is an attempt to overcome these ambiguities by analysing the efficiency and bias of LT as compared to HLC across different mosquito populations based on standardized mosquito sampling procedures.

4.3 Methods

4.3.1 Study sites

A set of data allowing direct comparisons of indoor LT to indoor HLC in multiple sites across Africa was compiled from selected data from two study platforms that conducted malaria transmission surveys. The platforms are the Malaria Transmission Intensity and Mortality Burden Across Africa (MTIMBA) and the Malaria Transmission Consortium (MTC). In addition, data from one independent survey carried out in Massavesse, Mozambique was included in this analysis. The MTIMBA surveys covered the years 2001–2004, and included sites in Burkina Faso (Oubritenga, Kourweogo and Nouna), Tanzania (Ulanga and Rufiji) and Ghana (Navrongo). The MTC covered the period 2009–2010, in sites in Zambia (Chisoba and Nyamumba) and Kenya (Aduoyo Minyare, Songo Rota, Kirindo and Kobala). All sites included in this analysis were of rural character (Table 1).

4.3.2 Mosquito collection

For each study, standardized mosquito sampling protocols were used. In the MTIMBA surveys, data included up to three years of daily indoor LT collections together with occasional HLC collections carried out for 48 nights of trapping (*i.e.* 24 periods of 2 consecutive nights of

collection) spread over a year. The standard procedure involved classification of the human population into geographical clusters of about 100 people who were living in the same area, based on each site's demographic data base. Each month, at least 30 people, referred to as 'index persons', were selected by simple random sampling from the database, and their respective clusters were enrolled in the survey. For the timing of mosquito collections, the selected index persons were distributed throughout the month, and for each index person, three additional people from the same cluster were randomly selected for LT for each collection night. The nearest compound to the index person was selected for indoor and outdoor HLC.

The selection of collection points in the Ulanga site deviated from the MTIMBA protocol. Here, the study participants were randomly selected from the Demographic Surveillance System (DSS) (Killeen, Tami et al. 2007). Villages in this database were initially subdivided into subvillages (similar to clusters) and the subvillages were then stratified into five strata based on mosquito net coverage per household. From this scheme each stratum was subdivided into five subvillages giving a total of 25 subvillages, that were assigned randomly to a week of sampling on a 25 week rotation (*i.e.* at 6 month intervals), allowing two visits per year for each subvillage. The sampling point within the subvillage was an index person that was randomly selected. For two consecutive nights, the household of a consenting index person was assigned a LT together with five consenting neighbouring households.

In each case, LTs were hung besides a sleeping place where one human volunteer slept covered by an untreated bed net. The LT was hung at the foot of the bed at about 1.5 m above the floor. The volunteers switched the LT on before going to bed, while the mosquito collectors switched the traps off in the morning

HLCs were done by volunteers that sat indoors and outdoors collecting mosquitoes which landed on their exposed limbs, using torchlight and aspirators (WHO 1975). At each sampling point, two pairs of volunteers conducted the HLC, with one pair replacing the other after the 6^{th} hour (e.g. 12 pm if catches started at 6pm). Within a pair, the volunteers interchanged

positions (indoors or outdoors) hourly. In the MTC study sites and the Ulanga site, only one pair of volunteers conducted HLC throughout the night, indoors and outdoors, without exchanging positions. Within each hour, they collected mosquitoes for 45 minutes and rested for 15 minutes (Killeen, Kihonda et al. 2006).

Occupants of these selected compounds were excluded from this exercise; houses were rented whenever it was necessary. Collection intensity and duration varied between sites. Additional data was provided from an independent survey carried out in Massavasse, Mozambique.

4.3.3 Protection of human subjects and ethical approval

Ethical clearance was obtained from respective local ethical review bodies. Participants were educated on the study procedures and were made aware of the health risks involved by their participation. As precautionary measure, study participants were screened regularly for malaria infection, followed by treatment of positive cases as per the local malaria treatment guidelines. In MTC study sites, volunteers were given malaria prophylaxis, Lariam® (Mefloquine) was provided in Kenya while, Malarone® (Atovaquone-Proguanil Hydrochoride) in Zambia.

4.3.4 Data analysis

The data were analysed by an extension of the method initially described by (Hii, Smith et al. 2000). Data for the mosquito species complexes used in the study, *Anopheles gambiae* sensu lato and *Anopheles funestus* sensu lato, were analysed separately. Only strata (collections by two methods matched by location and time) where at least one mosquito was captured by one of the sampling methods (indoor HLC and LT) were included in the analysis. The number of strata included in the analysis varied by mosquito species and site (Table 2).

In order to estimate the sampling efficiencies of the different methods, the following statistical model was used:

$$E(y_i) = \alpha_s E(x_i) \qquad (1)$$

where: $E(y_i)$ is the expected number of mosquitoes caught using LT in stratum *i*, $E(x_i)$ is the expected number of mosquitoes caught using the human landing method in the same stratum, *i*; α_s is the relative sampling efficiency corresponding to site *s*, compared to HLC for which the value is set to 1. The underlying mosquito density $E(x_i)$ is assumed to have a log-normal distribution, *i.e.* $\ln(E(x_i)) \sim Normal(\mu_s, \sigma_s^2)$, Poisson errors were assumed in the observed numbers of mosquitoes caught by any of the two methods so that: $x_i \sim Poisson(E(x_i))$ and: $y_i \sim Poisson(E(y_i))$ and the model therefore assumes the distribution of the numbers of mosquitoes caught by any method to be a log-normal mixture of Poisson distributions.

To allow for stochastic variation between sites, and to obtain an estimate of the overall average sampling efficiency across sites, the logarithms of the site-specific sampling efficiency, $\ln(\alpha_s)$, were assumed to vary normally about the overall average, $\ln(\tilde{\alpha}_t)$, *i.e.*: $\ln(\alpha_s) \sim Normal\left(\ln(\tilde{\alpha}_t), \tilde{\sigma}_t^2\right)$, thus leading to a hierarchical statistical model which was fitted using a Bayesian Markov chain Monte Carlo algorithm in the software WinBUGS version 1.4 (Spiegelhalter, Thomas et al. 2003). The parameters α_s, μ_s, σ_s, $\tilde{\alpha}_t$, and $\tilde{\sigma}_t$ were assigned weakly informative prior distributions which constrained them to be positive. Several different weakly informative prior distributions were explored.

To examine whether the sampling efficiency varied with the average mosquito density, the following extended model was also fitted:

$$E(y_i) = \left(\alpha'_s E(x_i)\right)^{\gamma_s} \qquad (2)$$

Where γ_s is an exponent corresponding to site *s.* A value of γ_s different from unity indicates a lack of proportionality between the mosquito sampling methods. In addition, α'_s will differ from α_s if γ_s is different from unity.

4.4 Results

Trapping efficiency of LT against HLC was analyzed across 13 different sites for *An. gambiae* s.l. and *An. funestus* s.l. separately. Useful information could be extracted from each stratum where (i) at least one mosquito was captured, and (ii) two trapping methods were deployed (Table 2). Those sites and species where more than 10 strata provided data were retained in the analysis. The number of sites included varied by species and trapping method (Table 2), with each site other than Rufiji being included in at least one analysis. Table 2 indicates the total numbers of mosquitoes thus included in the analyses for each site and mosquito species.

The most sampled species across sites was *An. gambiae* s.l. as sampled by both methods (Table 2). Linear models relating the number of mosquitoes caught in HL collections to those caught in the matched LT collections provided the average estimates the trapping efficiencies of the different methods, and addressed the question of whether trapping efficiency differs systematically between sites. This is illustrated in the forest plots (Figure 2) where a comparison of point and interval estimates from each site, as well as the estimate of the overall average trapping efficiency are displayed. LTs more often collected more mosquitoes than HLC, though this relation varies across sites. The overall sampling efficiency was $\tilde{\alpha}_t$ =1.07 [0.76-1.51] for *An. gambiae* s.l. and $\tilde{\alpha}_t$ =1.78 [0.90-3.44] for *An. funestus* s.l., corresponding to the dashed vertical lines in Figure 2.

Points in the funnel plot (Figure 3) are expected to form a triangular pattern centered on the best estimate of the average sampling efficiency, which corresponds to the vertical line. An asymmetric funnel would indicate a relationship between treatment effect and study size, suggesting either a selection bias or a systematic difference between smaller and larger studies. In Figure 3, a large proportion of the points fall outside the dashed triangle, indicating that there was much more variation between sites in the estimated sampling efficiency than was expected if the true value of the efficiency was the same in each site, but there is no

indication of any systematic bias either upwards or downwards in the averages, since there are points scattered either side of the vertical lines, more or less independently of the standard error.

Figure 4 and figures in the Appendix show the fitted relationship using equation (2) between both methods in the numbers of mosquitoes collected, whereby a straight line indicates a constant sampling efficiency with increasing mosquito density. Few of the curves for the individual sites are close to being straight lines (Figure 4, or appendix 1 and 2) and there was considerable variation among sites in the shapes of the curves. For *An. gambiae* s.l., the only sites in which the 95% interval estimates for γ_s included the value of one was Kobala, Kenya. Nine of the 12 sites analysed, the LT:HLC ratio increased as the number of mosquitoes increased (corresponding to $\gamma_s > 1$) (Table 2). For *An. funestus* s.l., the 95% interval estimate for γ_s included the value of one for Oubritenga, Kourweogo, Ulanga, and Massavasse, indicating that proportionality could not be excluded for these sites (Table 2). In three other sites , Nouna, Aduoyo Miyare and Navrongo, sampling efficiency of LTs increased with increasing mosquito density (*i.e.* the slope in Figure 4 increased as the density increased), but this increase was not significant. In the other three sites studied, the slope decreased as mosquito density increased. No consistent geographical pattern was observed in the efficiency of LT in sampling either species. Across densities, the relationship varied strongly even among sites that are close to each other, such as those located in Kenya.

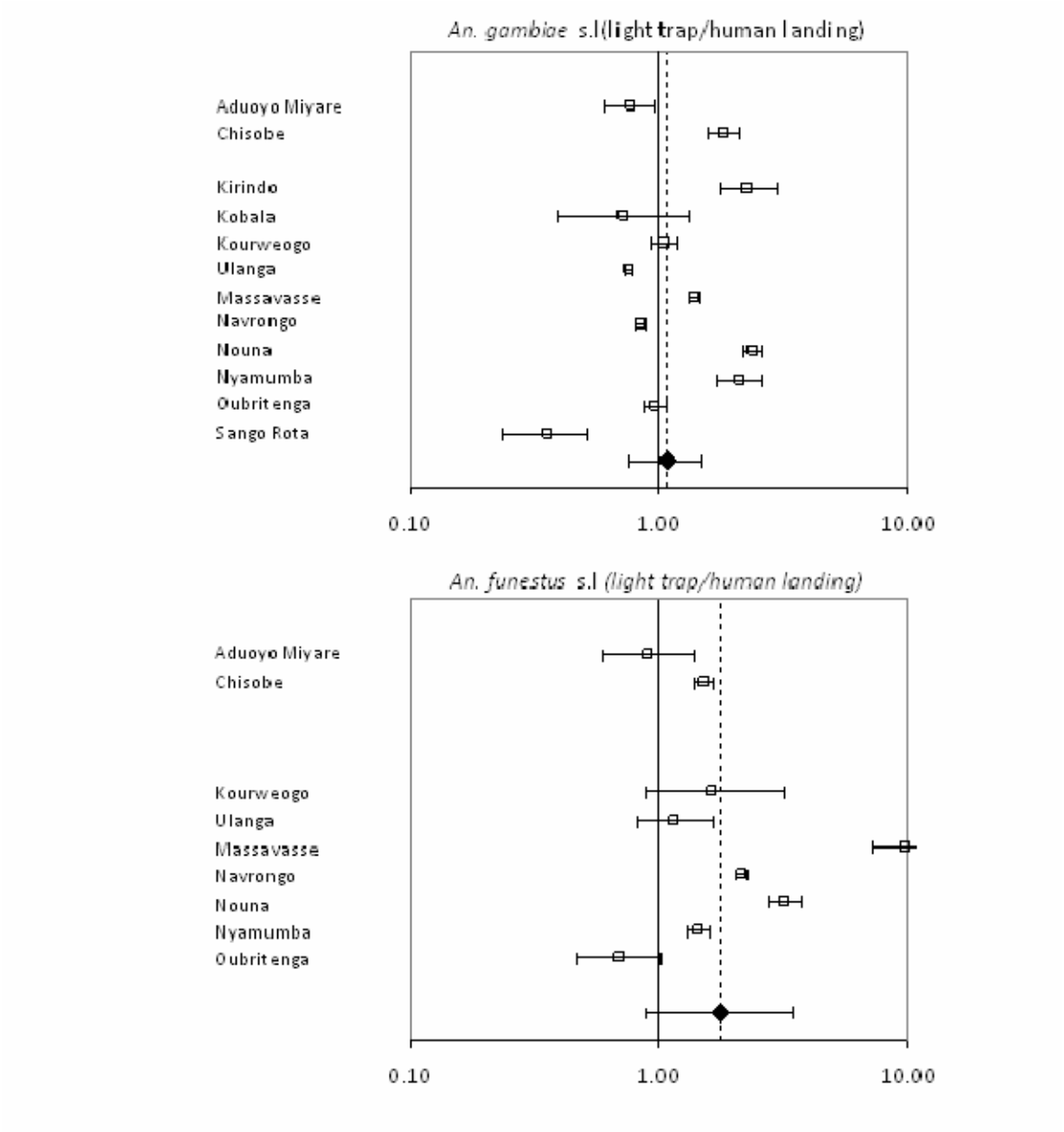


Figure 2 Forest plot giving the estimated sampling efficiency of light traps relative to landing collections, point estimates and 95% credible intervals of model 1., The margin is indicated by the dashed vertical lines which correspond to the best estimate of the overall average sampling efficiency.

Figure 3

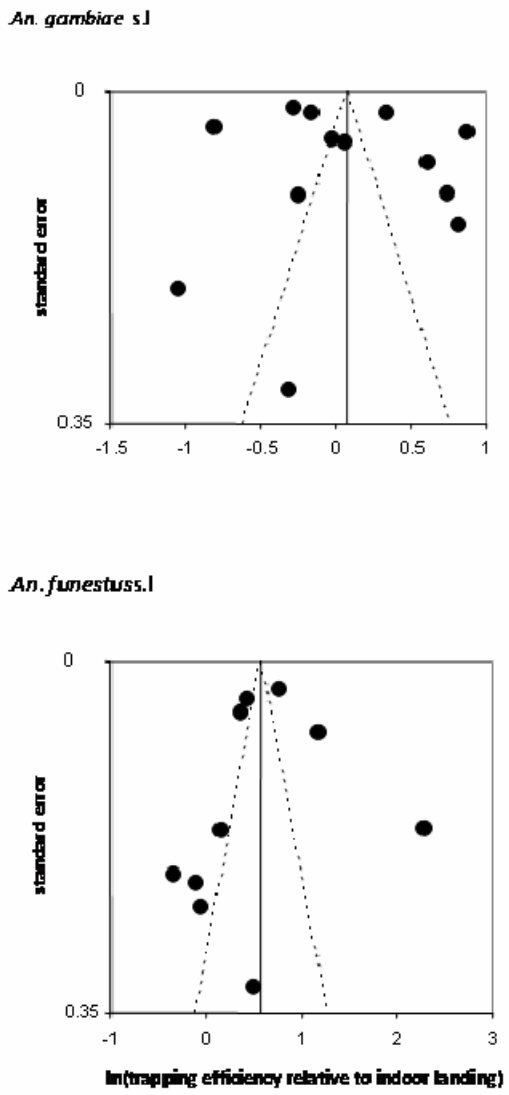


Figure 3 Funnel plots giving the estimated logarithm of the sampling efficiency for each site (horizontal axis), standard error (s.e.) of this estimate (vertical axis). The vertical line corresponds to the estimated overall average sampling efficiency. The dashed triangle corresponds to 95% pseudo-confidence limits calculated as 1.96±s.e. within which 95% of the points are expected to occur in the event that the differences between sites arise only because of sampling variation.

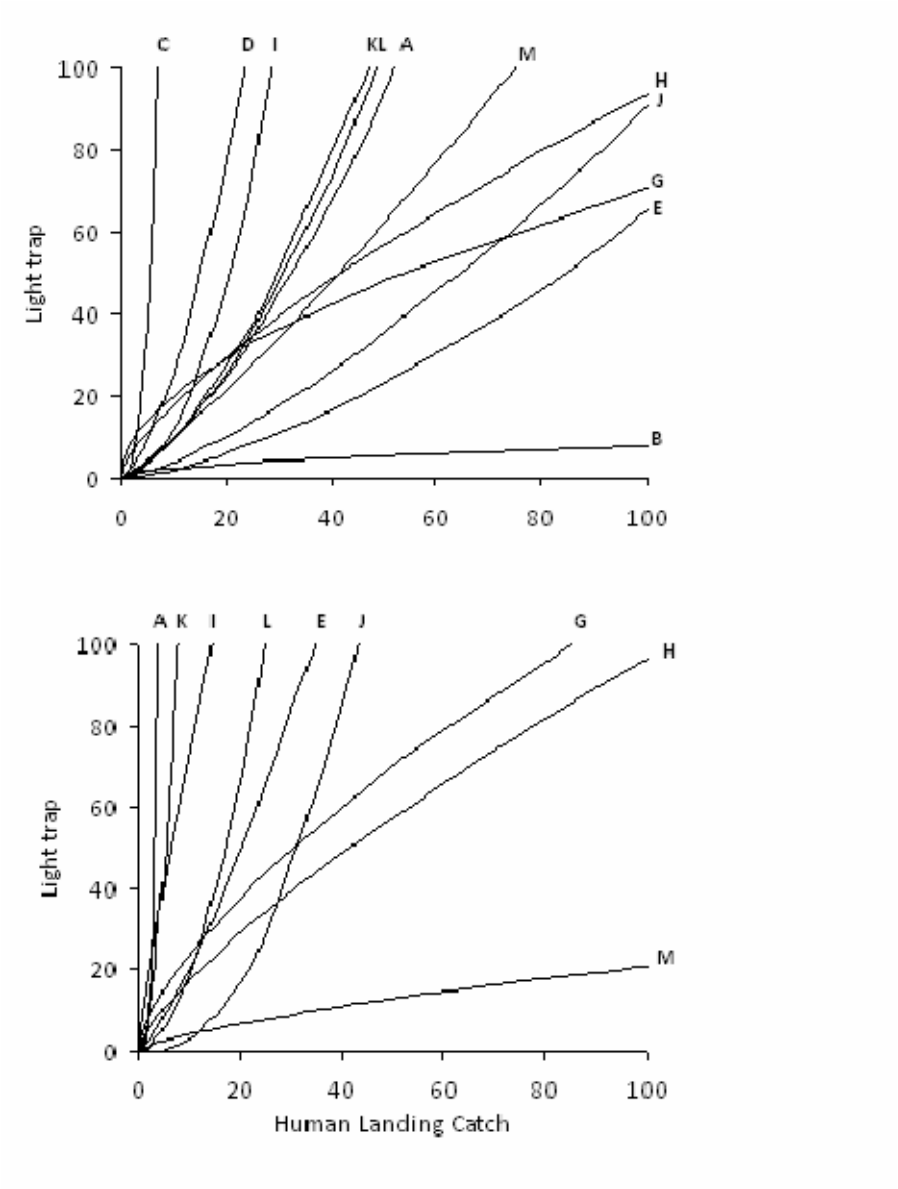


Figure 4 Fitted sampling efficiency as a function of landing catch. Lines correspond to field sites, as listed in table 1.

Table 1: Description of study platforms and their location, the column labelled as the site code serves as the key for figures 1 & 4.

Country	†Corresponding code in Figure 3	Site	Study platform	Duration	Vector population	
					An. gambiae s.l.	*An. funestus* s.l.
Kenya	A B C D	Aduoyo Miyare Sango Rota Kirindo Kobala	MTC	2009	*An. arabiensis*	*An. funestus* s.s.
Tanzania	E	Ulanga	MTIMBA	2004&2006	*An gambiae* s.s.	*An. funestus* s.s.
	F	Rufiji	MTIMBA	2001–04	*An.gambiae* s.s.	*An. funestus* s.s.
Zambia	G H	Chisobe Nyamumba	MTC	2009–10	*An. quadrianulatus*	*An. funestus* s.s.
Mozambique	I	Massavasse	Independent survey	2008-11	*An. arabiensis*	*An. funestus s.s.*
Ghana	J	Navrongo	MTIMBA	2001–04	*An gambiae* s.s.	*An. funestus* s.s.
Burkina Faso	K L M	Kourweogo Nouna Oubritenga	MTIMBA	2001–04	*An gambiae* s.s.	*An. funestus* s.l.

† Sites under survey were each assigned a code, after being ordered based on their proximity to one another.

MTC-Malaria Transmission Consortium

MTIMBA-Malaria Transmission Intensity and Mortality Burden across Africa

Table 2 Description of the sampling effort across sites, as well as the parameter estimates for model 1 and2.

Country	*Population (species / site)*	Total sampling nights included in analyses		Total mosquitoes included in analyses		Model (1)	Model (2)
		Light traps	Human Landing Catch Indoor	Light traps	Human Landing Catch Indoor	Site specific sampling efficiency (α_s)	Exponent testing proportionality (γ_s)
	An. gambiae s.l.						
Burkina Faso	Kourweogo	76	79	662	637	1.06 [0.95, 1.19]	1.52 [1.33, 1.69]
	Nouna	69	72	1834	812	2.40 [2.21, 2.62]	1.54 [1.45, 1.61]
	Oubritenga	109	111	771	791	0.97 [0.88, 1.07]	1.18 [1.06, 1.30]
Ghana	Navrongo	76	76	3316	3865	0.86 [0.82, 0.90]	1.37 [1.28, 1.45]
Kenya	Aduoyo Miyare	31	31	141	181	0.78 [0.62,0.97]	1.45 [1.15, 1.82]
	Sango Rota	32	32	31	87	0.36 [0.24, 0.52]	0.52 [0.28, 0.85]
	Kirindo	28	28	162	71	2.28 [1.76, 3.04]	2.78 [1.85, 4.17]
	Kobala	17	18	18	25	0.73 [0.39, 1.33]	1.59 [0.86, 3.70]
Tanzania	Ulanga	38	40	5013	9484	0.76 [0.73, 0.79]	1.49 [1.39, 1.59]
	Rufiji	6	6	27	24	NA	NA
Zambia	Chisobe	44	44	507	275	1.85 [1.59, 2.13]	0.54 [0.42, 0.65]
	Nyamumba	43	43	277	130	2.12 [1.72, 2.59]	0.72 [0.53, 0.98]
Mozambique	Massavasse	405	165	31673	3146	1.41 [1.34, 1.47]	2.04 [1.92, 2.17]
	An. funestus s.l.						
Burkina Faso	Kourweogo	33	33	23	14	1.63 [0.90, 3.24]	1.89 [0.77, 5.56]
	Nouna	50	52	819	267	3.24 [2.79, 3.74]	1.85 [1.67, 2.04]
	Oubritenga	59	61	41	56	0.70 [0.47, 1.03]	0.71 [0.31, 1.67]
Ghana	Navrongo	75	75	4373	2018	2.17 [2.05, 2.29]	2.33 [2.27, 2.38]
Kenya	Aduoyo Miyare	29	29	41	45	0.90 [0.60, 1.42]	3.57 [1.10, 100.0]
	Sango Rota	1	1	0	1	NA	NA
	Kirindo	5	5	1	4	NA	NA
	Kobala	3	3	4	1	NA	NA
Tanzania	Ulanga	30	32	65	98	1.16 [0.83, 1.66]	1.30 [0.44, 3.70]
	Rufiji	5	5	37	1	NA	NA
Zambia	Chisobe	52	52	1692	1101	1.53 [1.42, 1.65]	0.68 [0.60, 0.77]
	Nyamumba	55	55	938	648	1.45 [1.31, 1.59]	0.75 [0.64, 0.86]
Mozambique	Massavasse	207	50	9959	40	9.90 [7.29, 13.94]	0.87 [0.65, 1.19]

4.5 Discussion

Previous studies have provided evidence for the usefulness of LTs in estimating human biting activity as validated by comparisons with HLC collections conducted on the night before or after (Lines, Curtis et al. 1991; Mbogo, Glass et al. 1993; Fornadel, Norris et al. 2010) (Davis, Hall et al. 1995), but each of these considered only a single small geographical area, making it difficult to judge whether different results represent methodological differences or variations between sites in vector ecology. This study considers a large number of sites across different sites in East and West Africa and found that the relative sampling efficiency of LTs to HLC operating on the same night varied substantially.

LTs caught on average more anopheline mosquitoes than HLC, especially of *An. funestus* s.l., for which the LT sampling efficiency was higher than for *An. gambiae* s.l.. In seven out of nine sites, LTs sampled more *An. funestus* s.l. than HLC though in most sites, the numbers of mosquitoes caught by the two methods were not proportional. The LT:HLC ratio increased with mosquito density in nine out of twelve sites for *An. gambiae* s.l. and five out of nine sites for *An. funestus* s.l.. This suggests that the LT are sampling a different fraction of the mosquito population from the host-seeking component captured by HLC.

In several sites, LTs showed a propensity to reduced sampling efficiency as mosquito densities increased, therefore underestimating the density of host seeking mosquitoes and estimates of HBR. It is possible that at the time when these data were collected, the density of *An. funestus* s.l. was low in most of the sites, but LTs were generally more efficient in sampling this species at these density levels. Only in Massavasse (Mozambique) and Oubritenga (Burkina Faso) did LTs sample independent of the mosquito density. This implies that in these locations it may be valid to use LT alone to estimate *An. funestus* s.l. biting rates across seasons and mosquito densities by incorporating a simple calibration factor that accounts for the relative sampling efficiency relationship of LT to HLC, across mosquito density.

The relationship between LT and HLC for Ulanga has been previously reported by (Govella, Chaki et al. 2009), where extreme density dependence was illustrated graphically. This appears to be different to the density dependence relationship illustrated in this analysis. The discrepancy may be entirely due to different approaches used in displaying these mosquito data, where linear as opposed to curve models were fit on the (Govella, Chaki et al. 2009) analysis. Fitting a curve constraints data to go through (0, 0), the point of certainty, and the distance of (0, 0) from the rest of the data is dependent on the type of data transformation applied to the axis. In this analysis graphs presented in figure 5 allow for number of mosquitoes to be displayed, with the assumption that mosquitoes are behaving independent of each other, thus showing the amount of noise in the data as well as the dependence of individual points on a few mosquitoes. Essentially the findings of these two analyses are not contradicting each other but rather support the same finding that these two methods are not sampling the same components of the mosquito population in Ulanga area.

Widespread use of ITNs can influence mosquito behaviour by encouraging exophily and exophagy in the residual mosquito population (Govella, Okumu et al. 2010; Bugoro, Cooper et al. 2011; Russell, Govella et al. 2011). Therefore, some of the variation in sampling efficiency observed may be due to the intrinsic variations in the behaviour and species composition of the vector population caused by differences in the history of vector control among sites. For instance, preference of mosquitoes to rest indoors either prior or after feeding increases its chance of being sampled indoors by LTs.

Sibling species replacement within the *An. gambiae* s.l. complex has been reported to occur in some areas whereby *An. gambiae* s.s. (mostly anthropophilic, endophilic and endophagic) has been replaced by *An. arabiensis* (mostly zoophilic) (Bayoh, Mathias et al. 2010). Depending on enviromental conditions, *An. arabiensis* might go indoors to rest after blood feeding outdoors and then be lured by light from the LT and hence be caught in higher numbers in comparison to HLC. Thus, LT catches may include mosquitoes that were seeking a resting place after having fed elsewhere. These mosquitoes are likely to

be older (Mbogo, Glass et al. 1993), and more likely to be harbouring mature parasites if they were infected thus LTs have a potential to overestimate human exposure to mosquito bites.

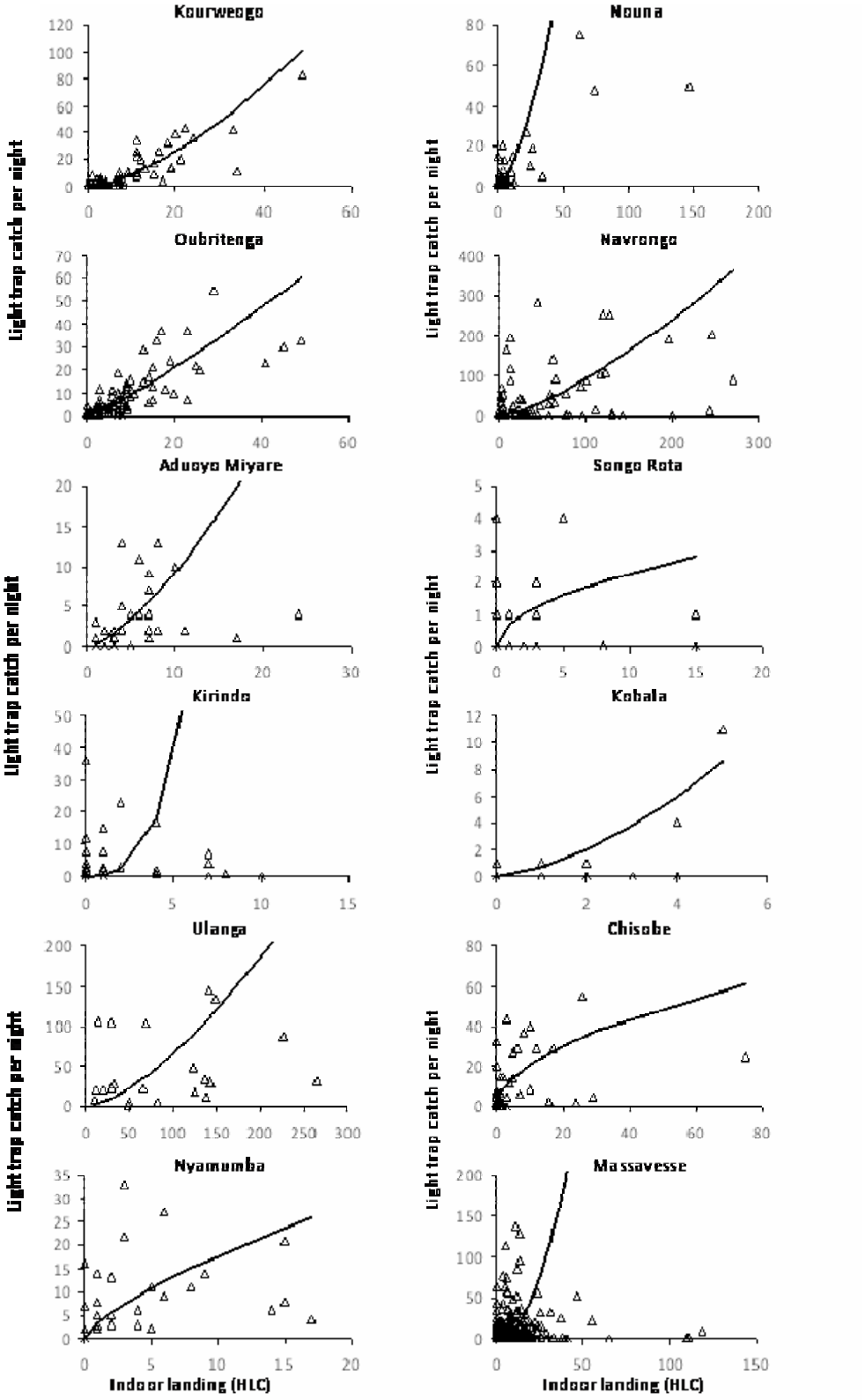
Using a similar approach to analysing data, Hii and colleagues, 2000 reported inconsistency in the efficacy of LTs across six species of the anopheline genera prevalent in Papua New Guinea (PNG). However, in PNG, the overall light trap catches were much lower than human landing collections. Nevertheless, these findings, support the conclusion that the sampling efficiency of light traps varies considerably geographically, even at a local scale (*e.g.* among sites within Burkina Faso and western Kenya).

Apart from previously mentioned factors, the importance of study design and adherence to the sampling protocol cannot be overlooked, especially in multi-site studies. This ranges from selection of households to positioning of LTs, and most importantly, supervision of the human landing catch that is naturally a very unpleasant exercise where people are likely to fall asleep especially in areas with low mosquito density. It should also be noted that outdoor HLC, though not analysed here, was performed as well, which may have influenced indoor catches.

Though LTs are convenient and more acceptable method of sampling mosquitoes, they have proved to be an inconsistent sampling tool that should be used cautiously with its limitations clearly borne in mind. The lack of proportionality of the two mosquito sampling techniques reduces the reliability of LT as an estimator of human biting rate across geographical areas, seasons, and various mosquito densities. It makes it impractical to devise a single calibration factor that could be used in estimating mosquito biting rates without the need of exposing volunteers to mosquito bites during HLC.

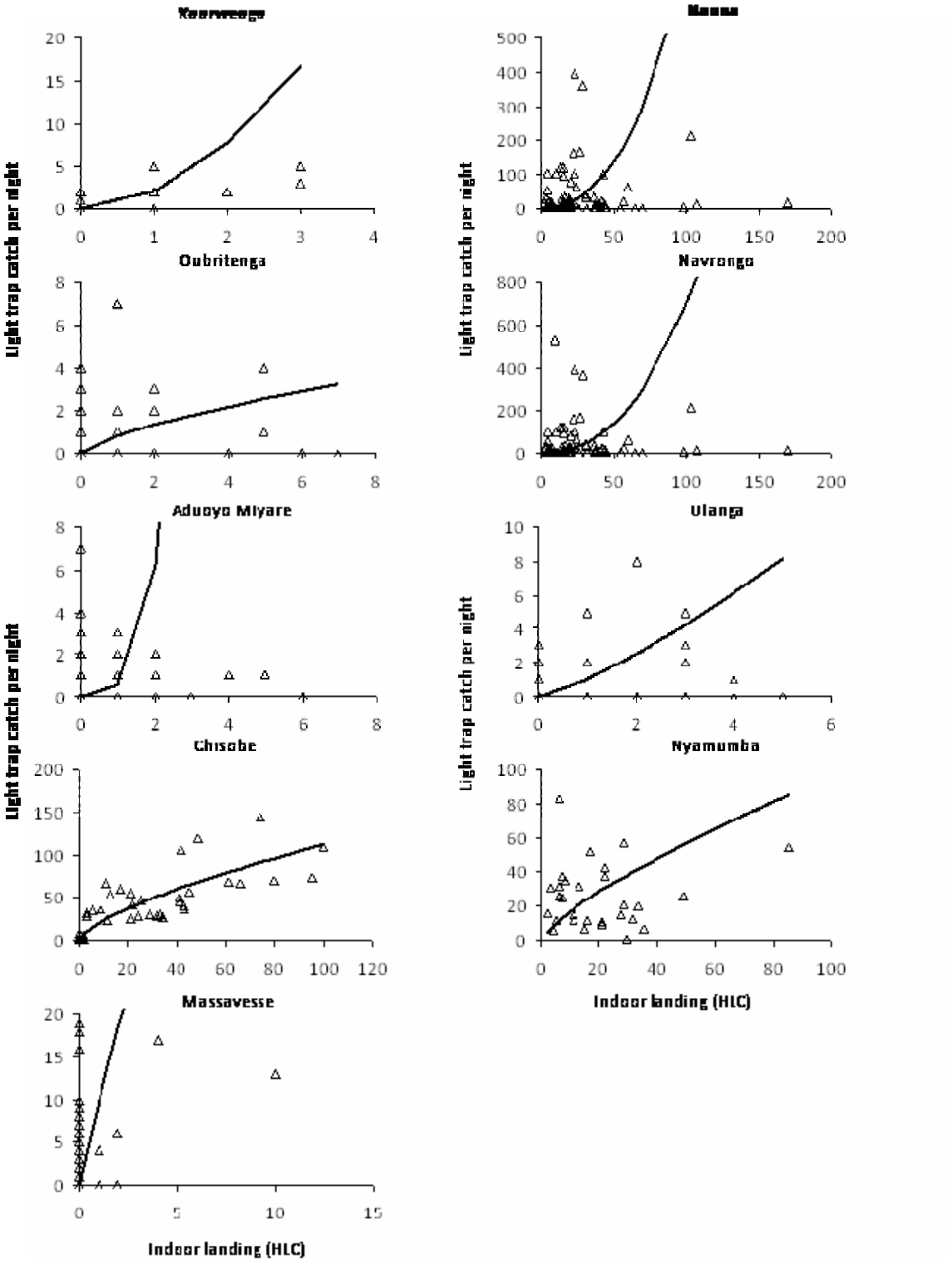
This necessitates the development of new tools that can sample representative numbers of indoor host seeking mosquitoes (Govella, Chaki et al. 2009), across seasons, mosquito densities and geographical area. Ideally, such a tool should be susceptible to bait bias by using standardized odour baits (Jawara, Smallegange et al. 2009), be easy to use and be within the same range of sensitivity as HLC and consequently adequate for monitoring

density trends as a safe substitute for HLC. On a local scale, if LTs are to be used in estimating HBR, then calibration with HLC during periods of both high and low mosquito density, also spanning the range of environmental conditions, should be done while awaiting development of new and better tools.



Appendix

Figure 5 Site specific plots of the number of *An. gambiae* s.l. sampled by LT against those sampled by HLC, the straight line describes the relationship based on model 2.



Appendix figure 6 Site specific plots of the number of *An. funestus* s.l. sampled by LT against those sampled by HLC, the straight line describes the relationship based on model 2.

5. Overall discussion

The results contained in this thesis span different sites, though they may lack in-depth characterization of mosquito species, provide an overall picture of mosquito mediated malaria transmission dynamics across malaria-endemic settings. However, based on models that utilize climatic data, the range and abundance of the two most prominent and important members of the *An. gambiae* complex, *An gambiae* s.s and *An. arabiensis,* can be predicted. These predictions indicate that *An. gambiae* s.s. was more likely to dominate wherever the two species were sympatric (Lindsay, Parson et al. 1998; Kiszewski, Mellinger et al. 2004). Such predictions together with other reported prevalence of mosquito species in these areas give an insight on what might have been prevailing in the mosquito populations, and therefore form a basis for further in-depth evaluations.

The need for broader analysis of mosquito population dynamics for baseline estimates of their taxonomic composition as well biting behaviour has been raised, particularly following the renewed goal of malaria elimination and eradication (Hay, Rogers et al. 2000; Kelly-Hope and McKenzie 2009; Shaukat, Breman et al. 2010). This goal has been followed up by large-scale roll out of vector control interventions such as ITNs/LLINs and IRS across malaria endemic areas. But, based on previous experience, mosquito populations have been reported to respond towards there interventions by either being sensitive or developing physiological or behavioral resistance towards the insecticides used in these tools. It is essential to characterize these mosquito populations for proper planning and execution of different vector control interventions. It is clear that most of human exposure occurs indoors but a residual minor fraction (11%) has been reported to occur outdoors (Huho, Briet et al. 2013; Lindblade 2013). Existence of such residual exposure to malaria transmitting mosquitoes needs to be monitored as it can reduce the success of these interventions (Molineaux, Shidrawi et al. 1979). Systematic characterization of malaria transmission dynamics across diverse transmission intensities, may aid in development of realistic plans towards achieving success in

malaria control. There is need to select key parameters that can be monitored longitudinally across mosquito populations together with standardized guidelines on how to carry out such surveys preferably on selected sentinel sites (Ranson, N'Guessan et al. 2011; Gatton, Chitnis et al. 2013). All these efforts linked with generic templates of data entry, centralized data storage and access might serve to generate real-time information on the response of mosquitoes to ongoing interventions. Experts in the field can use such database to influence malaria control policy by reporting prevailing scenarios and extrapolating possible scenarios that may emerge based on application of mathematical models.

5.1 Development of standardized methods for measuring malaria transmission

In other fields of malaria research, there are published protocols on how to carry out surveys and measurements of different malaria transmission parameters (Bousema, Stevenson et al. 2013). This area is not well developed in the area of malaria entomology, where there is need for establishing standard sampling procedures together with standardized data entry schema (Russell, Kiware et al. In preparation). This will allow cross-site analysis and real-time meta-analysis of malaria transmission dynamics. The study procedures should clearly outline the reasoning behind choice of sampling points, allocation and placement of mosquito traps, time of collection and frequency of mosquito collections (as determined by the intensity of malaria transmission). Associated descriptions of study sites studies, including the vector ecology, land use patterns, human population demographics and behaviour, use of malaria interventions and their coverage levels, weather patterns and malaria parasite rates in the human population and elevation need to be shared. Procedures, rules regulations of data access, sharing and dissemination should be clearly outlined at the initial stages of the study. Such data platforms can also be useful for validation of malaria transmission predictive models and for meta-analysis of the impact of interventions on malaria transmission.

Though a standard procedure for sampling adult mosquitoes was developed for all the MTIMBA sites, deviations from it were noted even at a country level. These discrepancies

might have been as a result of lack of co-ordination between the different sites. In the course of this study I had a chance to design and implement a protocol for monitoring adult mosquito population in Bagamoyo, Tanzania. This protocol was further modified to be included and tested across sites as a means of providing baseline measures of malaria transmission dynamics across sites that were participating in the phase III evaluations of Rts,S candidate malaria vaccine (Appendix 1). The protocol was also adjusted to include a cross sectional survey of malaria parasite rates in the human population.

5.2 Methodological challenges

5.2.1 Sampling schemes

In Tanzania there were differences between the study sites of Kilombero-Ulanga (*the two districts were treated as one study site*) and Rufiji. They both adhered to randomizing selection of households, but followed different sampling schemes. Annual rounds of non-repeated sampling were done in Rufiji while in Kilombero-Ulanga bi-annual repeated stratified sampling was used.

Lack of adhering to standardized procedures, or having procedures that do not clearly stipulate their implementation might have accounted for the variability observed in the initiation times for sampling adult mosquitoes. Comparison of efficiency of LT and HLC in sampling adult mosquitoes would require the two methods to operate under similar conditions as possible. While operation of light traps requires well charged batteries that can run for the whole night, HLC require keenness of collectors for the whole sampling period. Ideally LT and HLC should start at similar time but this was not the case across the sites reported in this study. Though it was found that delays in the initiation of HLC in some sites may not have overestimated significantly the estimates of indoor human exposure reported, but it might have influenced the total number of mosquitoes caught per method. Across sites it was becoming apparent that there are fractions of mosquito populations that bite ether early in the evening or early in the morning, likely to be missed if sampling does not include these times.

5.2.2 Estimation human infectiousness

Oocyst prevalence was determined on blood fed mosquitoes that were collected resting indoors, including those that managed to enter inside the mosquito nets of appointed household members, either as a consequence of improper hanging of the mosquito net or due to having other entry points such as holes. These mosquitoes were kept for 2 to 3 days to allow digestion of the blood meal, and then were dissected to reveal the gut and stained with mercurochrome for ease of observation of oocysts under a dissecting microscope. These procedures were done in the field, but given the intensity of transmission and the high number of mosquito catch that can arise in such areas, dissections of mosquitoes to observe the presence of oocyst might have been limited in comparison to the density of adult mosquitoes collected. It is then likely that during periods of high transmission intensity, an underestimation of oocyst prevalence can be reported, calling for close supervision of field teams. Rarely in the field, there were instances when field workers deviated from standardized procedures, by dissecting mosquitoes in normal drinking water instead of using normal saline. Such can lead to destruction of mosquito tissues therefore obscuring clear visibility of oocysts. At times sampled mosquitoes that were set aside to digest their blood meal, were observed to be given plain water instead of sugar solution. It is likely that such a situation may induce physiological stress as a result of sugar deprivation, which may directly or indirectly influence the number and quality of oocysts produced.

Despite these shortcomings, we report a significant increase in oocyst rates in the the site where gametocyte reducing artemisin based combination therapy was used for treatment of uncomplicted malaria. This validates the sensitivity of this approach in measuring human infectiousness in areas of high malaria transmission intensity (Haji, Smith et al. 1996), but raises a question on its sensitivity when applied in areas of low malaria transmission intensity. Given the global agenda of eliminating malaria transmission intensity, areas of declining malaria transmission intensity are emerging across malaria endemic areas (Alonso and Tanner 2013). Therefore there may be a need

for more sensitive molecular based assays for determination of the presence of the initial stages of the parasite inside the mosquito.

Recently an assay for quantification of *Plasmodium falciparum* infections in the mosquito vector has been developed and tested in laboratory experiments of human infectiousness (Bell and Ranford-Cartwright 2004). Ideally these assays should allow large number of mosquitoes to be processed and be sensitive enough to detect infections at low levels with measurable error margins (Bell and Ranford-Cartwright 2004). Furthermore, such assays can reduce the level of supervision that may be required when carrying out large-scale evaluations of levels of human infectiousness on residual mosquito populations following application of malaria interventions (*particularly* those with a transmission blocking effect). Malaria transmission blocking vaccines are in the initial phases of development (Dinglasan, Armistead et al. 2013). Given promising results, latter phases of its evaluation will require large scale clinical trials of its effectiveness. At such a stage, one of the key measurable endpoints of the efficacy of this intervention is likely to be the prevalence and intensity of oocysts in local population of mosquitoes, ideally with an ability to discern the different strains of malaria parasites. This will require representative sampling of mosquito populations and supervision of field teams on storage of living mosquitoes in the field while awaiting dissection. This is due to high chances of predation mainly from ants upon collected mosquitoes, if they are not kept propely, this can easly be overcome in the field by careful set up of ant-traps. A standardized assay that can be done, either in the laboratory or in the field on whole mosquito will be of great significance in this area of research.

5.2.3 Measurement of parasite rates in the human population

It is also possible that the prevalence of gametocytes on a co-joined study in the intervention site, reported an underestimated level of gametocytes. Though given the high intensity of malaria transmission, the underestimation may be less when compared to area of low transmission intensity according to a recent meta-analysis of the efficiency of these two methods (Okell, Ghani et al. 2009). Detection of gametocytes based on

microscopy can be supplemented with molecular methods, especially where levels of malaria transmission intensity are low (Okell, Ghani et al. 2009). Even though no detectable changes of gametocyte prevalence were recorded post ACT roll out in our study (Khatib, Skarbinski et al. 2012), the limitation of the method used to determine the presence of gametocytes implies that there may have been even higher levels of gametocytes circulating in the human population.

5.2.4 Measurement of human biting rates

To reduce the bias that can be introduced by human biting rates obtained based on HLC, rotation of volunteers between indoor and outdoor compartments can be done, as well as introduction of half night shifts whenever possible or short breaks (*no more than 15 minutes*) so as to reduce fatigue among mosquito collectors (Gimnig, Walker et al. 2013). A recent study has revealed that volunteers of HLC had 96.6% lower incidence of malaria in comparison to non volunteers (Gimnig, Walker et al. 2013). Such a finding implies that whenever necessary, HLC can be used to estimate human biting rate, given that there is well managed care of volunteers by provision of malaria prophylaxis together with regular follow-up for incidence of malaria.

Estimation of human biting rates should account for the presence of personal protection measures for a given area, especially where coverage levels are high (>60%) and the overall effects on these interventions on mosquito survival of mosquito population become more evident (Killeen, Smith et al. 2007). All night HLC may overestimate the mosquito-human biting rate in areas where it is common to use personal protection measures (Filion, Paul et al. 2006). In such a case estimates of this parameter can be taken to imply the maximum level of human exposure that can occur for that given area, a useful tool when designing implementation of malaria control interventions.

Care should be taken in selection of households where mosquito collection is to be done, depending on the objectives of the study; some variables may introduce a bias such as presence of cattle or a breeding site. In the absence of strict field supervision and study procedures that have a self correcting mechanism e.g. recording geo-position of

sampling points, it is also more likely for field workers in long-term surveys to build familiarity and to sample households that are easier to reach as opposed to farm houses that are usually far and not easily accessible in rural Africa. Presence of cattle may introduce a competitor to the human bait, especially in areas where malaria transmission is carried out by opportunistic vectors such as *An. arabiensis*, *An. rivulorum*, *An. parensis* (Wilkes, Matola et al. 1996). In principle randomization of sampling points, may reduce the effects of these local variations on measured human exposure estimates.

5.3 Emerging needs in monitoring malaria transmission intensity

- Tools for measuring malaria transmission intensity when it occurs at low levels are lacking. Such scenarios are becoming more common following the success of vector control, and more sensitive methodologies that can measure and monitor malaria transmission at these levels need to be developed.
- Currently, measurement of outdoor host seeking mosquitoes can be done using pit traps, clay pots, ramp traps, resting searches odour baited traps (Mukabana, Mweresa et al. 2012) as well as a Furvella trap that has been reported to sample mosquitoes that are considered exophagic in Mozambique (Kampango, Cuamba et al. 2011). While these tools need to be tested in different epidemiological settings, the need for estimation of the relative contribution that residual outdoor malaria transmission is becoming more in demand. However HLC remains to be the gold standard for estimating outdoor host seeking mosquitoes.
- There is scarcity of detailed descriptions of mosquito populations of medical importance across Africa, given the commitment of combating malaria and reducing its burden in this region. However, much success has seen in the past decade towards reduction of malaria burden in Africa (WHO 2011). For continued success there might be a need to characterize mosquito populations in parallel to the massive scale up of vector control interventions. This can be done by regular monitoring of mosquito biting behavior, together with tracking for signs of either physiological or behavioral

resistance towards insecticides that are being used. As has been suggested by other researchers, establishment of sentinel sites for monitoring these dynamics need to be done, together with a long-term commitment for longitudinal follow-up of mosquito population dynamics (Ranson, N'Guessan et al. 2011; Gatton, Chitnis et al. 2013).

- A set up that allows continual monitoring of malaria transmission dynamics can reduce the level of unprepared ness when signs of reduced efficacy to the current interventions start to appear. Learning from past experience, where malaria transmission persisted in some areas due to development of resistance among the residual mosquito population towards IRS programs (Molineaux, Shidrawi et al. 1979; Brooke, Kloke et al. 2001; Snow, Amratia et al. 2012), such populations are more likely to be composed of opportunistic species, which can utilize both humans and other vertebrates as a source of blood meal depending on the ratio and availability of hosts in the population (White 1974). These strains flourish in reduced competition from previously dominating anthropophilic mosquito species that came into contact with the insecticide indoors were sensitive enough for the insecticide to cause mortality. Human availability becomes the driving force in the biting pattern of surviving strict anthropophilic mosquitoes such as *An. gambiae*. Depending on the malaria transmission dynamics, the amount of residual malaria transmission can be controlled by combination of implementation of zooprophylaxis, larval control together with improved housing into the use of ITNs/LLINs and IRS (Kirby, Ameh et al. 2009; Fillinger and Lindsay 2011; Achee, Bangs et al. 2012). Different target product profiles can be evaluated for their role in reducing outdoor malaria transmission (Killeen and Moore 2012). Lack of alternative vector control measures of these residual mosquito populations, as well as well as long-term commitment towards malaria control, these mosquito populations can revert back to their initial composition. Mathematical models can be useful in predicting the possible maximum protective efficacy of different combinations of interventions for a given malaria transmission scenario.

- Human blood index represents the proportion of mosquito blood meals that are obtained from humans (Garrett-Jones 1964), there is a need to keep monitoring this parameter in mosquito populations following selection pressure that can be introduced by successful vector control interventions. This estimate of mosquitoes may change based on proportionate availability of human and other vertebrate hosts. At high coverage of personal protection measures, strict anthropophiles such as *An. gambiae* s.s. and *An. funestus* s.s. may decrease in number or shift their biting patterns to times when humans are more likely to be unprotected (Charlwood and Graves 1987; Magesa, Wilkes et al. 1991; Bogh, Pedersen et al. 1998; Bayoh, Mathias et al. 2010; Pappa, Reddy et al. 2011). Measurement of human blood index among residual mosquito populations can aid in understanding the role of different mosquito species in malaria transmission dynamics of a given area (Animut, Balkew et al. 2013), despite being opportunistic *An. arabiensis* was found to maintain high preference of human blood after the introduction of ITNs in Zambia (Fornadel, Norris et al. 2010).
- Regular characterization of mosquito populations following up scaling of personal protection measures may lead to incrimination of subtypes of malaria vectors, e.g. the discovery of an exophilic line of *An. gambiae* s.s. in Burkina Faso (Riehle, Guelbeogo et al. 2011), or exposure of previously insignificant mosquito species (Stevenson, Laurent et al. 2012).

Appendix

Measurement of Malaria Transmission Intensity in study areas implementing the Multi-Centre Phase III Rts,S Malaria Vaccine Efficacy

GENERAL INTRODUCTION

1. BACKGROUND

Malaria is one of the most serious health problems facing the developing world. Despite enormous and diverse efforts to control this disease, it is still among the top three most deadly communicable diseases (Sachs and Malaney 2002). Current estimates by the World Health Organization (WHO) indicate that 40% of the world's population is at risk, while 300-500 million infections are reported per annum leading to mortality estimates that range between 0.7 and 2.7 million (WHO/UNICEF 2003; Snow, Guerra et al. 2005). About 90% of malaria related deaths in the world occur in Sub-Saharan Africa (Hay, Guerra et al. 2004; Snow, Guerra et al. 2005). Aside from the human tragedy, an economic disaster may be inevitable for malaria stricken countries (Gallup and Sachs 2001). Gallup and Sachs (2001) reported that in the period between 1965 and 1990, the annual economic growth rates in malarious countries was 1.3% lower compared to non-malarious countries. Conversely, this disease can be made worse by social-economic issues such as dramatic population growth in the face of weak public health systems, new agricultural practices such as irrigation, dam construction and climate change (Hay, Noor et al. 2002; Sachs and Malaney 2002).

The major vectors of this disease are mosquitoes in the *Anopheles gambiae* Gillies *sensu lato* group, a complex of seven subspecies distinguished by cytotaxonomic and molecular

means (Coluzzi, Sabatini et al. 2002). This complex of vectors is responsible for approximately 80% of global malaria and morbidity that occurs in sub Saharan Africa (White, 1974). Two members of this complex are responsible for the majority of malaria transmission in Africa: *Anopheles gambiae* Gilies *sensu stricto* and *Anopheles arabiensis* (White 1974). These species are mostly sympatric in their distribution although the latter is more distributed in arid areas (Lindsay, Parson et al. 1998). Apart from malaria, these species are important carriers of *Wucheria bancrofti,* a filarial worm.

Malaria control in Africa is mainly reliant upon anti-malaria chemotherapy and insecticide based vector control strategies. However, drug resistance in parasites has undermined the efficacy of this approach (White 1999; Howard, Scott et al. 2003). Insecticide-based vector control is also threatened by the emergence and spread of resistance to the compounds used (Hemingway, Field et al. 2002; Yawson, McCall et al. 2004). As a result, new approaches and strategies must continually be developed if the fight against malaria is to be won. Vaccines have historically been one of the most cost-effective widely accepted and easily administered means of controlling infectious diseases. Recent breakthroughs have proven that a malaria vaccine could contribute to the control of malaria. Although several candidate vaccines are under development, only one called RTS,S/AS02A that targets the pre-erythrocytic has proven efficacious in a highly endemic sub-Saharan African setting (Alonso, Sacarlal et al. 2004; Alonso, Sacarlal et al. 2005). RTS,S/AS02A targets the pre-erythrocytic stages of this parasite. In addition to giving personal protection to the recipients, such a vaccine might also reduce the transmission of malaria lowering their infectiousness to mosquitoes, particularly in areas with an entomological inoculation rate (EIR) of 10 infectious bites per year or less (Killeen and Smith 2006). Malaria transmission intensity conventionally expressed as the EIR, is a quantity describing the average number of infectious bites one individual will typically receive each year in the community. In some locales in Africa, the EIR may reach over 1000 bites per person annually (Beier, Killeen et al. 1999). EIR values may vary depending on environmental and demographic conditions such as rainfall, vegetation cover, human-population density and land use patterns.

2. RATIONALE

RTS,S/AS02A targets the pre-erythrocytic stage of this parasite and will soon be tested in 10 African sites in a phase III trial. The objective of the present study is to include EIR estimates to allow better extrapolation and interpretation of the estimates of efficacy of this vaccine trial. EIR provides an estimate of the exposure to malaria in terms of the number of sporozoite positive mosquitoes biting an average person per year. This is typically accomplished by sampling female *Anopheles* mosquitoes using light traps hung besides intact mosquito nets and appropriate sampling schemes can allow estimation of community mean exposure levels. Specifically our goal is to provide background EIR estimates for study sites implementing a phase III trial of RTS, S/AS02A by conducting conventional evaluations of mean community-level EIR. This study will serve as a valuable point of reference for evaluating the efficacy of this malaria vaccine as a function of the wide range of the transmission intensities typical of participating endemic setting.

3. GOAL AND OBJECTIVE

3.1. Overall Goal

To estimate the level of transmission by anopheline mosquitoes in the study communities over a five year period by determining the Entomological Inoculation Rate (EIR) in study areas implementing phase III vaccine trial.

3.2. Objectives

- To estimate the overall transmission level of study populations in the phase III of RTS, S/AS02A trial.

- Seasonality of vector abundance
- Comparison with historical data
- Determination of principal vectors of mosquitoes

4. METHODS

4.1. Study area

This study is based on the extensive work conducted in by the MTIMBA project.

4.2. Sampling procedure (EACH SITE WILL NEED TO ADD A SITE SPECIFIC DESCRIPTION (ADENDUM)

A list of heads of households will be made for each village/hamlet/compound **(CHANGE ACCORDING TO THE SETTINGS OF YOUR STUDY SITE)** in the study area with the help of village health care workers **(CHANGE ACCORDING TO SETTINGS OF YOUR STUDY SITE)**. The listing of heads of households will be assembled based on lists provided by local government representatives at *kitongoji* (sub-village) level. These names will then be entered on the computer using Microsoft Excel or Access by following the order of proximity of the villages to one another. A sequence number in that based on the same order will be assigned to each head of household. This list in its wholesome will comprise a sampling cluster. To allow representative monitoring of transmission across all villages in the study area, every month a list of households to be sampled will be randomly selected from the enumerated cluster of heads of households.

Selection of houses will be based on random numbers generated by computer program e.g. Microsoft Excel. The list of random numbers will then be merged with the names in the cluster. Randomization will be such that there will be more than one option (the first option being given a priority) of households to be sampled at any given day of field work. This will be done as a precaution for cases of migration or death of the head of household selected. The entomology field workers will then be provided with a copy of

the list of households to be sampled each with sampling options and references of how to locate the selected houses within the cluster. In order to enable logistically feasible but methodologically rigorous sampling, the field team will work their way through the monthly sampling list in order of proximity, covering the full list in a geographical sequence at their own discretion which minimizes travel to and between sampling points.

Typically, this means that the field team will work from a common mobile base camp within the study area **(ADAPT TO LOCAL CIRCUMSTANCES, INFRASTRUCTURE AND PRACTICES SO LONG AS THE LIST IS REPRESENTATIVELY SAMPLED AND THE FIELD PROCEDURE IS PRACTICALLY FEASIBLE)**, fanning out to sample a set of nearby houses chosen from the sampling list. This sampling scheme will be implemented such that, on each of the four nights of every week, every entomology field worker will set a minimum of 3 traps so that at least 12 houses are sampled resulting in single night catches of no less than 48 houses per week. This leads to a minimum of 192 households/light trap nights to be sampled from the cluster monthly. Houses will be geo-referenced with a GPS. To allow consideration of differences in housing type and their effects on indoor mosquito densities, a questionnaire identical to that used in the **(MTIMBA OR MTC CHOOSE ACCORDING TO PREFERENCE AND APPROPRIATENESS)** study (see appendix D) will be filled for each house to described its' key structural features, such as open eaves, ceilings etc. and the use of relevant domestic vector control measures, such as ITNS will be noted.

4.3. Light trap collections

Sampling will be done indoors by the use of CDC light traps, fitted with incandescent bulbs and laid close to a human volunteer sleeping under a bednet in his/her usual sleeping place, in order to estimate anopheline biting rates. The CDC light traps will be installed at about 1.5m above the floor next to the foot of the bed of the person (see appendix B). Those with an untreated or treated net will be left with their own nets,

while a project net (Olyset long-lasting insecticidal net (SUBSTITUTE LOCALLY COMMON FRONT LINE LLIN-THIS HAS MINIMAL EFFECT ON CDC-LT CATCHES) will be used for the person when one is not already available. Light traps will be operated from sundown to sunrise in each house and bags will be emptied every morning. The time the light trap is set up and taken down should also be recorded. Use of personal protection against mosquitoes should be recorded for each house (e.g. type of net (Olyset, Net Poa, (SUBSTITUTE MOST LOCALLY COMMON AND RELEVANT ITN BRANDS), Other), treated or untreated nets, mosquito coils and local repellents). It will also be recorded if cooking is occurring in the room being sampled. The occupants of the house will be instructed to switch on and off the traps. A label indicating the date, name of head of household number will be placed in the bag. Mosquitoes will be will be counted and sorted by species at a central point in the field (MAY BE IN A LAB AT SOME SITES), and stored in 10 ml tubes with a small quantity of silica gel in the bottom, separated from mosquitoes with a small amount of tissue paper or cotton wool. Samples will be labeled with the unique collection ID consisting of the enumeration area (EA), cluster (SUBSTITUTE LOCALLY RELEVANT TERMS OR OMIT IF NONE) (CR), household number (HH), date (DT), collection ID (CO), Taxon (TX) and mosquito class (MC). Samples should be stored in a freezer and checked regularly to ensure that they are both dry and frozen. Mosquitoes will be transported from the field to the laboratory in a cool-box.

Mosquitoes will subsequently be tested as follows:

(i) Head and thorax tested for CSP by ELISA. If necessary (high mosquito numbers and low sporozoite rates), these may be conducted on pools of 10 or less mosquitoes from single collections.

(ii) A sample of 1000 *An. gambiae s.l*. will be identified to species by PCR.

Note that all mosquitoes analyzed in this way will receive and be stored in a tube labelled with a unique ID consisting of the above mentioned ID for its collection plus an individual mosquito number.

4.4. Calibration of light traps using comparative human landing catches.

The sensitivity and sampling efficiency of the light trap catches (LTC) used will be estimated by calibration against human landing catches (HCL), by comparing indoor and outdoor human landing catches with LTC traps in randomly chosen houses, distributed across the study area.

The calibration exercise will involve all field staff, conducting one all night HLC for one night of every week. They will collect mosquitoes which land on their exposed legs using torchlight and test tubes or aspirators. The first pair will work for 6 hours from 6pm to until 1 am, and the second pair will takeover from 1 am to 7 am. For each hour 45 minutes will be spent catching mosquitoes, leaving 15 minutes of break time. Collections will be made from 6pm to 7am with the mosquitoes caught during each hourly period labeled and stored separately. The house to be used will be chosen from the list household to be sampled for that particular day, the selection criteria being the first house in the list to consent their participation to this exercise. Routine mosquito collection (HLC & LTC) for the calibration of LTC, will be therefore be performed in the same house at the same time to maximize comparability.

4.5. Human activity cycle.

This will be determined by interviews done by the entomology team who will fill in a form for the neighbor of one of the houses where they had set the traps, they will record which people are active hourly until everyone goes to bed (see appendix D) on the evening when the light traps are set up. Similarly on the morning when the traps are removed, an observer will record which people are active hourly from a house in the

same area from 05.00 until everyone gets up or 07.00 if the last person to get up is after this time (see appendix D).

5. ANALYTICAL PLAN

EIR as a measure of transmission intensity will be obtained by multiplying the average biting rate of mosquitoes by their sporozoite prevalence. The average biting rate represents the average number of mosquito bites that one is exposed to per night; it will be computed by dividing the total number of mosquitoes caught by LTC over the whole sampling period divided by the total number of sampling trap nights in that sampling period. Sporozoite infection prevalence is a proportion, calculated as the number of mosquitoes in the catch that tested positive for the presence of sporozoites in their salivary glands divided by the number of mosquitoes tested for sporozoite presence over the whole sampling period. The efficiency of light traps against the gold standard of human landing catch will be adjusted by the calibration factor obtained by dividing the average catch of HLC by the average catch of LTC. Estimates of transmission level of the different study populations will be obtained by the determination of their respective sporozoite rates multiplied by the biting rate of mosquitoes in that locale. Thus mean EIR estimates for each village **(CHANGE ACCORING TO THE SETTINGS OF YOUR STUDY SITE)** in the whole area targeted for phases III will be obtained as described above. This information will simplify the selection and stratification of the phase III study area and allow paired comparisons to be made among enumeration areas **(SUBSTITUTE LOCALLY RELEVANT TERM, EG VILLAGES)**, clusters **(SUBSTITUTE LOCALLY RELEVANT TERM)** and neighboring even households with very different transmission intensities.

6.0. ETHICAL CONSIDERATIONS

6.1. Informed consent

Permission for conducting the study will be sought from the district and local authorities **(REPLACE WITH LOCALLY RELEVANT AUTHORIZING AUTHORITES)**. Prior to their recruitment individuals for human landing catch will be given information about the purpose of the study and what is to be involved. These participants will be informed of all potential risks and benefits before being invited to participate but it will be made clear that they are free to refuse participation or to withdraw from the study at any stage. Their informed consent will be sought and in addition informal individual consent will be required prior to setting traps in households.

6.2. Risks to participants

The primary foreseeable health risks risk involved with participation in this study is the possibility of being infected with malaria while conducting a human landing catch. Participants are protected from biting mosquitoes when trapping is conducted with CDC-light traps, particularly if an long lasting insecticidal net is provided by the project, but the human landing catch method consequently the method inherently carries an increased risk of malaria infection for which we will provide precautionary health services.

The participants in HLC will be informed of the purpose, duration and procedures of the study, the potential risks involved and the precautionary health services to be provided to them as participants. Informed consent will be documented using the attached form (see appendix C) and participants will be allowed to withdraw at any stage. All HLC participants will be screened for malaria parasites by microscopic examination of a Geimsa-stained thick smear blood sample and at any time that they exhibit possible symptoms or request screening. Any participants found to be positive for malaria parasites during the study will be provided with standard first line treatment, specifically

Co-Artem (Artemether-Lumefantrane) and, in case of recurrence, will be referred to a nearby health facility for appropriate second-line treatment and medical care.

6.3. Dissemination

The results of this study will be disseminated to all levels of the district and local authorities upon completion of the study. The results will also be disseminated to the international community through publication in international peer-review journals.

APPENDIX A

FILL IN APPROPRIATE INFORMATION BELOW IS AN EXAMPLE ADAPTED FROM THE BAGAMOYO SITE

INVESTIGATORS

Salim Abdulla and Bernadette J. Huho

Ifakara Health Research and Development Centre, Bagamoyo Research and Training Centre, P.O Box 74, Kiko Avenue, Mikocheni B, Dar es salaam.

LITERATURE REVIEW ON THE TOPIC SPECIFIC FOR YOUR COUNTRY / SITE:

In Tanzania malaria accounts for 30% of the national disease burden; where 14-18 million malaria cases are estimated annually. The greatest burden of disease occurs in children under 5 years and pregnant women; who account for 7.1 million and 1.69 million cases respectively each year (MOH 2002). The major vectors of malaria in Tanzania are in the *Anopheles gambiae s.l* complex, particularly *An gambiae s.s* and *An arabiensis* both of which are widespread at all times of the year over the whole country except on a few zones (Clyde 1967).

SITE DESCRIPTION:

This study will take place in Bagamoyo district in conjunction with clinical trials of RTS,S/AS02A malaria vaccine. It will cover all the villages that will be involved in phase III trial of this vaccine trial covering about 1000 square kilometers. The area is surrounded by the Indian ocean on the eastern side and Ruvu river on the west and northern borders with an uninhabited forest reserve on the southern border. Approximately 81,000 of its people are village inhabitants with most of the villages being reachable by dirt road; and within a 30 minute drive. The main rainy season is from March to May, with a second period from November to December, although occasional rains occur at all times of the year. The average rainfall is 1,200 to 2,100 mm per year. The vegetation is characterized by year-round grassland vegetation with subsistence agriculture being practiced throughout the study area. A total of fifteen villages will be sampled in this study.

APPENDIX B

PROCEDURES FOR ESTIMATING EIR.

This is a set of minimum guidelines to assist in the implementation of the EIR estimation protocol. In addition equipment required for mosquito catches is listed below.

Before one starts collecting mosquitoes, a form should be prepared to record the following details (if available) about each enumeration area (EA), cluster **(SUBSTITUTE LOCALLY RELEVANT TERMS OR OMIT IF NONE)** (CR), household number (HH), date (DT), and ID of the person who sleep under the net, the time the traps was set up and the time is was stopped. Number of mosquitoes caught by species, the number of mosquitoes caught over the course of the night, whether

treated or untreated net was present in the house, ITC, spraying or coils were used etc.

Procedures for CDC light traps.

i. Select house by way of randomization as in protocol
ii. Mount the aluminium plate by slotting it in to the space/hole provided on top of the CDC trap.
iii. Fix the netting bag round the bottom base of the CDC trap.
iv. Moisten some cotton wool and put in one of the side pockets of the net.
v. Place an identification label in the other side pocket of the net.
vi. Using a hook or otherwise attach the 2 meter string in the hole on top of the trap.

From a suitable position in the roof rafts, ceiling or wall, hang the trap by means of the string at a level of 1.0 – 1.5 meters above the ground (the highest point being the aluminium plate).

i. Connect the negative pole of the battery to the negative lead of the trap (blue plug or silver wire) and the positive pole to the positive wire (red plug or copper wire) at the required time at dusk (e.g. 6 pm).
ii. Make sure that your CDC trap is blowing downwards into the netting by placing a wet hand/ finger above and below the fan to feel the wind direction.
iii. At dawn (in the morning e.g. 6 am) when you come to collect the trap, first tie the neck of the netting with a piece of string while the trap is still operating
iv. Disconnect the battery terminals and dismantle the trap if you need to take it back otherwise just take the batteries for recharging.
v. In the laboratory, place the netting with the mosquitoes in a freezer at -20ºC for about 10 minutes to immobilize the mosquitoes and empty the contents on a white sheet of cloth or paper before transferring then into a suitable cup or storage facility for later identification by morphology. The mosquitoes can also be collected alive in the field from the netting with the help of a pooter or

sucking tube and transferred into paper or plastic cups which is them transported in a cold box to the laboratory for identification.

Common Problems associated with the operational use of CDC light traps and their remedy:

i. The wind blowing upwards instead of downward. In which case you should reverse the connection to the battery terminals.
ii. Bulb giving a dull light. The battery must be dull and you should change or recharge the battery fully.
iii. Mosquitoes drying up quickly after entering the trap. Place a moistened cotton wool in a petridish or suitable container inside the netting before fixing it to the trap.
iv. Fan too noisy. Apply a suitable lubricant to the motor.
v. Hole in the netting. Seal with a tape or sow it with a needle and thread.

P.S. You can improvise mosquito-collecting cups by punching a hole in the lid of a stool pot (Sterilin) to the size of the pooter or sucking tube diameter and plug the hole with some cotton wool.

B) Procedures for human landing collection.

Human Landing sampling exercise from a single house indoors and outdoors:

i. Label all your cups for indoor and outdoor catches with catchers details, date, location and hour of collection or use a code numbering system of your own indicating the hours collection.
ii. Position one man inside the room selected for HLC (as in the protocol) sitting on a chair with the trousers rolled up to the knee and another man at least 20 meters away from the indoor man's house, sitting outside on a chair with the trousers rolled to the knee.
iii. With the help of a pooter/sucking tube/test tube and a touch light, catchers should collect all the mosquitoes that land on their legs or arms during the period they are doing the exercise.

iv. Time the collection from dusk to dawn on hourly basis (changing the collection cups every hour).

v. Replace the catchers with the two rested members of the team at 1 am.

vi. Store all completed cups collected in a cold box before they are taken to the base laboratory for identification of the mosquitoes.

vii. Ensure that every four weeks, all four catchers have conducted each of the four shift-catching station combinations for one night.

P.S. If the test tube method of collecting landing mosquitoes is preferred or more feasible then these can be plugged with cotton wool directly, labeled with the collection details and stored in a cold box before being transported to the base laboratory for identification.

Possible problems encountered during HLC: -

i. Exposures to wild infective mosquitoes. Administer prophylactic anti-malarial (Artemether-Lumefantrane) as per prescription.

ii. Catchers sleeping during collections. Do a regular supervision of catchers and offer them coffee, tea and food to help them keep awake.

C) Checklist of equipment

1) **Checklist of equipment for LTC:** -

1. Aluminium plate
2. Body of the CDC trap
3. Netting for CDC trap
4. Charged Batteries 6V
5. Battery carrying box
6. Hook
7. 2 meter String
8. Spare bulb
9. Mosquito storage cups / paper cups with netting covers
10. Sucking tube
11. Battery charger
12. Marker pens
13. Cotton wool
14. Cold box with some ice packs

2) Checklist of equipment for HLC:-

1. Sucking tube or pooter

2. Mosquito collection cups
3. Cotton wool
4. Cold box with some ice packs
5. Touch light
6. Batteries
7. Spare bulbs
8. Alarm clock / watches
9. Marker pens
10. Chair
11. Anti-malarial prophylactic drug.
12. Coffee, tea and food

APPENDIX C

INFORMED CONSENT

INFORMED CONSENT INFORMATION TO THE HEAD OF THE HOUSEHOLD.

Malaria is a major disease in Tanzania (**CHANGE ACCORDINGLY)**. Malaria is transmitted through the bites of anopheline mosquitoes. Knowledge of the species and densities of anophelines mosquitoes in an area forms a good basis for their control. In this study we a trying to find out the type, when and where people in the study area most likely to be bitten by malaria vector mosquitoes. If you consent that your house be used in this study, the research team will visit your house and place mosquito traps in them morning. If you agree to participate in this study, we request you to sign this sheet to affirm your consent.

Yours Sincerely

Principal investigator.

INFORMED CONSENT RECORD FORM FOR THE HEAD OF THE HOUSEHOLD

I, …… (Household head) clearly understand the aims of the project that intends to measure malaria transmission intensity in Bagamoyo (**CHANGE ACCORDINGLY)** and I agree to my household being in the study.

Signature (Household head) _____ Date _____

Address_____
_____
_____
____

Witness:_____ Signature_____
Date_____

Investigator: _____ Signature
_____Date_____

TRANSLATE PRECEEDING INFORMED CONSENT FORM TO NATIVE LANGUAGE IF NEED BE BELOW IT IS AN EXAMPLE IN SWAHILI

TAARIFA YA UTAFITI KWA MKUU WA KAYA

Malaria ni ugonjwa unaoongoza hapa Tanzania, unaambukizwa kwa kuumwa na mbu anayeitwa anopheles. Uchunguzi na utambuzi wa aina ya mbu na idadi ya anopheles husaidia katika kupanga mikakati na mbinu za kuzuia na kuangamiza mbu hao. Katika utafiti huu tutajaribu kuangalia aina na wakati gani na wapi watu wa sehemu hii huweza kuumwa na mbu. Iwapo utatoa idhini ya kukubali nyumba yako itumika kwenye utafiti huu, watafiti watakutembelea na kuweka mitego ya mbu ndani ya nyumba yako na kuukusanya mtego huo kesho yake asubuhi.

Kama unakubali kushiriki katika utafiti huu, tunakuomba uweke sahihi kwenye fomu hii kuonyesha tumepata ridhaa yako.

Mtiifu

Mtafiti.

IDHINI YA KUKUBALI KUSHIRIKI KWENYE UTAFITI KWA MKUU WA KAYA

Mimi……(Mkuu wa Kaya) Nimeelewa madhumuni ya Mpango huu wa “kupima maambukizi ya Ugonjwa wa Malaria na ninakubali nyumba yangu itumike kwenye utafiti huu.

Sahihi (Mkuu wa Kaya)
_____Tarehe_____

Anuani_____
_

_____
_

Shahidi:_____Sahihi_____Tarehe_____
_

Mtafiti:_____Sahihi_____Tarehe_____

INFORMED CONSENT INFORMATION FOR THE HUMAN LANDING CATCH PARTICIPANT

Malaria is a major disease in Tanzania (**CHANGE ACCORDINGLY)**. Malaria is transmitted through the bites of anopheline mosquitoes. Knowledge of the species and densities of anophelines mosquitoes in an area forms a good basis for their control. In this study we a trying to find out the type, when and where people in the study area most likely to be bitten by malaria vector mosquitoes.

As a participant you will be you will be required to use human landing catch method which entails you to catch mosquitoes as they attempt to bite you and may increase your risk of acquiring malaria. You will therefore be provided with screening for malaria parasites and offered treatment if found to be infected. If you agree to participate in this study, we request you to sign this sheet to affirm your consent.

Yours Sincerely

Principal investigator

INFORMED CONSENT RECORD FOR THE RESEARCH ASSISTANTS

I, ……. clearly understand the aims of the project entitled of measuring malaria transmission intensity in Bagamoyo (**CHANGE ACCORDINGLY)** and I agree to participate in the study. During my participation in these studies, I understand that field mosquitoes can infect me with malaria parasites. I also understand that carrying out man-landing catches may expose me to increased risk of infection with malaria. I therefore undertake to submit to screening for malaria parasites by microscopic examination of thick film blood smears. I also understand that I am entitled to free treatment for malaria if found to be infected with malaria parasites. I understand that I may revoke my consent and leave the study at any stage.

Name of entomology field worker: _____

Name of entomology field worker: _____Date_____

Witness Name: _____

Witness signature: _____Date_____

TRANSLATE PRECEEDING INFORMED CONSENT FORM TO NATIVE LANGUAGE IF NEED BE BELOW IT IS AN EXAMPLE IN SWAHILI

IDHINI YA KUSHIRIKI KATIKA UTAFITI – MTAFITI MSAIDIZI

Malaria ni Ugonjwa unaoongoza hapa Tanzania, unaambukizwa kwa kuumwa na mbu anayeitwa anopheles. Uchunguzi na utambuzi wa aina ya mbu na idadi ya anopheles husaidia katika kupanga mikakati na mbinu za kuzuia na kuangamiza mbu hao. Katika utafiti huu tutajaribu kuangalia ainia na wakati gani na wapi watu wa sehemu hii huweza kuumwa na mbu na vilevile kufanya majaribio ya njia mpya za kugundua idadi na aina ya mbu waliopo katika sehemu maalum.

Kama mshiriki utatakiwa ktumia njia ya kukamata mbu wakati wanapotaka kukuuma na hii inaweza ikasababisha hatari ya kupata Malaria, hivyo utapatiwa msaada wa kuchunguzwa vimelea katika damu na kupewa matibabu iwapo utakutwa na ugonjwa wa Malaria.Kama umekubali kushiriki katika utafiti, tafadhali tunaomba uweke sahihi katika fomu hii kuonyesha ridhaa yako.

Mtiifu

Mtafiti.

Mimi…….. nimeelewa madhumuni ya mpango wa kuchunguza mwenendo wa maambukizi ya Ugonjwa wa Malaria Bagamoyo na ninakubali kushiriki katika utafiti huu. Wakati wa kushiriki kwenye kukamata mbu wanaweza wakaniambukiza Malaria kwa kuniuma. Vile vile naelewa na kufahamu kuwa wakati ninapokamata mbu kwa kuniuma itaniweka kwenye hatari ya maambukizi ya Malaria na kwa hiyo ninakubali kupimwa damu kwa ajili ya kuchunguzwa vimelea vya malaria na kutibiwa iwapo nitakutwa na ugonjwa.

Nafahamu kuwa zoezi hili ni la hiari na halimzuii mtu kujitoa iwapo ataamua kufanya hivyo wakati wowote.

Jina la Mtafiti Msadizi_____

Sahihi ya Mtafiti Msaidizi_____Tarehe_____

Jina la Shahidi_____

Sahihi ya Shahidi_____Tarehe_____

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