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Integrated malaria vector control in different agro-ecosystems in western Kenya

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Malaria, Anopheles gambiae, An. arabiensis, An. funestus, Plasmodium falciparum, Bacillus thuringiensis var. israelensis, Gambusia affinis, arrow roots, source reduction

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Human malaria is a complex disease and its severity is a function of the interaction between the Anopheles mosquito vector, the Plasmodium parasite, the hosts and the environment. A two-year longitudinal study on the population dynamics and breeding characteristics of local mosquito species and malaria prevalence was undertaken in different agricultural settings within two highland sites (Fort Ternan and Lunyerere) and a peri-urban area (Nyalenda) in western Kenya. Community perception and knowledge on malaria, causes of malaria and the control of mosquito vectors were established through a questionnaire. This information allowed for the development of small-scale mosquito larval control strategies combining source reduction, environmental manipulation through provision of shade and biological control using predatory fish (Gambusia affinis) and application of the bio-larvicide, Bacillus thuringiensis var. israelensis (Bti). The main malaria vector species, Anopheles gambiae Giles sensu stricto and An. arabiensis Patton, were both present in all sites as larvae, while An. funestus Giles was only recorded in the highland villages. The majority (86%) of the mosquito breeding sites encountered were a result of human activities. Plasmodium falciparum, the main malaria parasite in the region, was observed with no significant differences in malaria prevalence among the study sites. Malaria was regarded as a burden by the community members who expressed willingness to take part in mosquito control. Field trials targeting mosquito larval control demonstrated the feasibility of environmental and biological control methods in man-made, mostly agricultural, mosquito breeding habitats.

Introduction

Human malaria is one of the most serious public health problems in sub-Saharan Africa, where it kills between one and two million persons every year, mostly children under five years of age and pregnant women (Snow & Marsh 2002). In much of tropical Africa, Plasmodium falciparum is the dominant and most serious malaria parasite, responsible for high degrees of morbidity and mortality. Most of sub-Saharan Africa has stable endemic malaria because climatic conditions ideal for transmission coincide with the ranges of the malaria vectors. In East Africa the main malaria vectors are the mosquitoes Anopheles gambiae Giles (figure 1), An. arabiensis Patton, and An. funestus Giles. Recently, An. coustani Laveran has been documented as a potential malaria vector in East Africa (Geissbühler et al. 2009).

Environmental conditions – such as rainfall, temperature, humidity – and topography, including altitude, exposure and latitude, determine the intensity of malaria risk, expressed as hypo-, meso-, hyper- and holo-endemicity (Gilles & Warrell 1993). Extreme climatic events such as El Niño or excessive rainfall may contribute to unusual epidemics (Bouma et al. 1994).

Small increases in temperature in areas that experience low temperatures (highlands) can result in serious outbreaks where the population has developed little or no immunity (Patz et al. 2000). In stable malaria situations, transmission levels are characteristically high with little variation between years, and collective immunity in the population is also high, thus epidemics are unlikely to occur. Here, prevalence rates are characteristically high, with most severe cases in infants. In unstable situations, transmission levels of infections vary from year to year and collective immunity is low, therefore, there is a high potential for epidemics. Low immunity (non-developed or impaired immunity) is the most significant predictor of malaria in those infected with P. falciparum and this phenomenon largely restricts the epidemics to areas with low herd immunity, which mainly occur in the highlands in the tropics (Thomson & Connor 2001).

High-altitude areas in East Africa experienced infrequent outbreaks of malaria between the 1920s and the 1950s (Garnham 1945), which was attributed to anomalous warming in the African region (IPCC 1998). From the 1960s to the early
1980s, malaria was not reported in the Kenyan highlands after a malaria eradication campaign. However, since 1988, frequent malaria epidemics have occurred here at elevations higher than 1,500 m above sea level (Malakooti et al. 1998, Hay et al. 2002), and by the year 2001, the epidemics had spread from three to fifteen districts over a period of thirteen years (Githeko & Ndegwa 2001). In western Kenya, *P. falciparum* is the main parasite causing malaria. The primary malaria vectors include species in the *An. gambiae* complex and *An. funestus*. The two species of local importance in the *An. gambiae* complex are *An. gambiae* sensu stricto (henceforth termed *An. gambiae*) and *An. arabiensis*.

Climatic conditions, temperature, altitude, humidity, rainfall, flooding and phenomena such as El Niño can work towards either increasing malaria infection by creating more mosquito breeding habitats, or decreasing malaria infection by splashing out water collected in small pools, thereby eliminating mosquito breeding habitats. Climatic conditions influence the development, reproduction and survivorship of anopheline mosquitoes and malaria parasites (Zhou et al. 2004). At Londiani, situated at 2,286–2,377 m above sea level, *An. gambiae* is present but the mean temperature never reaches the critical figure for effective transmission of the parasites (Gilles & Warrell 1993). The domestic habits of *An. gambiae* could be the contributing factor to its survival at such heights. *Anopheles gambiae* mosquitoes spend most of their life in human habitations, which are 3-5 °C warmer than the prevailing outside temperatures (Garnham...
1945, Bodker et al. 2003, Koenraadt et al. 2006). These indoor conditions may prove conducive to transmission in these otherwise Plasmodium-hostile environments (Reiter 2001). A model developed by Zhou et al. (2004) predicts that during an epidemic, the most severe malaria cases come from highlands’ human populations, which have not been regularly exposed to malaria infections.

**Biology of anopheline malaria vectors in the highland areas**

Mosquitoes (Diptera, Culicidae) of the *An. gambiae* and *An. funestus* complexes transmit the majority of malaria cases in sub-Saharan Africa. *Anopheles gambiae* and *An. arabiensis* are widespread over the African continent, although the latter species extends into more arid areas (Coetzee et al. 2000). Observations of the larval habitats of *An. gambiae* have noted a preference for temporary, sunlit pools (Gillies & De Meillon 1968, Gillies & Coetzee 1987), whereas *An. arabiensis* appears to exploit permanent, artificial habitats such as rice fields (Githeko et al. 1996a). However, consistent differences in habitat use by *An. gambiae* or *An. arabiensis* have not been observed and both species have often been found occupying small, sunlit, transient habitats (Minakawa et al. 1999, Gimnig et al. 2001, Koenraadt et al. 2004). Populations of *An. arabiensis* survive the dry season better, whereas populations of *An. gambiae* peak shortly after onset of the rainy season (Githeko et al. 1996a). In Tanzania, *An. arabiensis* has been reported to be common during the short rains and just before the long rains, whereas *An. gambiae* pre-dominate during and just after the long rains (White et al. 1972). *Anopheles gambiae* is highly anthropophilic, meaning that it almost exclusively bites humans, whereas *An. arabiensis* is more opportunistic, meaning that it may feed on humans as well as on animals, depending on the relative availability of hosts (Githeko et al. 1996b). *Anopheles funestus* has a similar distribution over Africa as *An. gambiae* but it prefers to breed in large, permanent bodies of water with emergent vegetation (Gillies & De Meillon 1968, Klinkenberg et al. 2003) and populations generally peak after those of *An. gambiae*.

In the Kenyan highlands all the malaria has been largely caused (i.e., mediated) by *An. gambiae* and a typical epidemic usually begins in late May or early June, following the ‘long rains’, reaches its peak in June or July and is on the decline by August, with year-to-year variability. *Anopheles gambiae* shows increase in density only after the long rains, whereas *An. funestus* density is seen to vary in direct proportion to the proximity of the permanent breeding grounds rather than rainfall (Gar- nham 1929), but their habitats do not differ much in the adult stage. In Mumias, a high-altitude site and a large-scale sugarcane growing zone in western Kenya, Shililu et al. (1998) found malaria transmission intensity to be low but perennial, with the main vectors being *An. gambiae* and *An. funestus*, both highly anthropophilic. Balls et al. (2004) in their study in the Usambara Mountains, Tanzania, reported that altitude played an important role in determining malaria infection due to its effect on temperature. It is possible that temperature simply governs vector densities through a direct physiological effect on larval development time (Bodker et al. 2003, Paaijmans et al. 2009).
In the Amani hills in Tanzania, deforestation is thought to have created new habitats for effective vectors, and the elimination of shade produced a marked change in local climate (Reiter 2001), whereas in the Ugandan highlands, the elimination of papyrus has created a habitat for *An. gambiae* and *An. funestus*, leading to increased transmission (Lindblade et al. 2000). *Anopheles gambiae* were collected in greater proportions along cultivated swamps and transmission was entirely due to this species. The increased malaria transmission has been attributed to increased local temperatures near cultivated swamps, combined with occasional excessive precipitation.

**Problem definition**

Human malaria is a complex disease and its severity is a function of the interaction between the *Anopheles* mosquito vector, the parasite, the hosts and the environment. Malaria control typically targets *Plasmodium* parasites – the dominant and most serious protozoan parasite, causing severe malaria in human beings in sub-Saharan Africa – or *Anopheles* vectors (see Box 1). Parasite control is now being undermined by the rapid spread of parasite resistance to once-effective antimalarial drugs (Trape 2001). It is therefore unrealistic to make malaria control exclusively dependent on clinical care. The most effective method of malaria control remains the interruption of mosquito-host contact (Lindsay et al. 2002) through vector control strategies. Vector-directed control strategies have mainly targeted adult mosquitoes through indoor residual spraying (IRS) with potent insecticides and/or insecticide-treated bed nets (ITNs) (Robert et al. 2000, Schellenberg et al. 2001, RBM 2005). However, the rapid development of insecticide-resistant mosquitoes (N’Guessan et al. 2007, Ramphul et al. 2009) undermines current malaria control strategies and opens the way for introduction of alternative control measures.

One alternative method would be through mosquito larval control as mosquitoes spend a considerable part of their life in the aquatic stage (Box 1, figure 2a, b). However, for any larval control strategy to be effective, the ecology of the local
vector in terms of the species responsible and characteristics of breeding habits needs to be known. The research presented here was a longitudinal study undertaken to establish the temporal and spatial dynamics of malaria mosquito larvae, adult mosquitoes and malaria prevalence in three environments in western Kenya, and to analyze the feasibility of environmental and biological methods for mosquito larval control. In addition, a social study was carried out to find out the knowledge and perceptions of the community members towards malaria, its causes and their willingness to take part in control activities.

Two highland villages (Lunyerere and Fort Ternan) and one peri-urban (Nyalenda) area next to the city of Kisumu were selected (figure 3). The selected villages provided a good comparison of malaria-endemic sites. Although at almost similar altitude levels, the two highland villages are significantly different in terms of topography, land use and associated anthropogenic activities. The peri-urban lowland site selected provided a good example of how an increase in the urban population in Kisumu city and the need for food security for the expanding population has encouraged small-scale irrigated agriculture. Fort Ternan (figure 3a) agriculture is an old practice that has not changed over generations, whereas Lunyerere and Nyalenda (figure 3b, c) represent transformed swamp areas that are currently used for agriculture.

Key findings and discussion

Larval and adult mosquito vector dynamics

In Western Kenya, Anopheles gambiae, An. arabiensis and An. funestus are the principle vectors of human malaria (Coetzee et al. 2000). Anopheles gambiae prefers to breed in open sun-lit, shallow pools of water, whereas An. funestus prefers more permanent bodies of water with emergent vegetation (Gillies & Coetzee 1987, Klinkenberg et al. 2003). The findings of the study indicate that 86% of all the mosquito breeding habitats recorded were man-made. The most productive (in terms of mosquito larval density) man-made habitats were drainage canals, hoof prints, tire tracks, rice paddies and watering taps (either leaking or broken) with a constant water flow. Anopheles arabiensis was the main species in Nyalenda, consistent with the reported distribution range of this sibling species; it prefers to breed in low and hot areas as opposed to An. gambiae, which has been found to prefer cool, high-altitude areas (Coetzee et al. 2000). Contrary to this expectation, based on larvae collected, An. arabiensis was the most abundant (71%) anopheline species in Fort Ternan, setting a new record in the Western Kenya highlands. Although larvae of An. arabiensis were frequently collected in this area, only one adult mosquito was collected from indoor sampling during a 24-month period. Previous studies on larval development in Fort Ternan indicated that the larvae of both An. gambiae and An. arabiensis rarely complete their development, mainly due to low temperatures (Koenraadt et al. 2006). In Lunyerere, the main adult mosquito vector was An. gambiae; An. arabiensis made up 12.5% of the total An. gambiae s.l. collected. Previous studies in the same highlands did not report the presence of An. arabiensis (Githeko et al. 2006, Ndenga et al. 2006), hence this was the highest percentage to be ever recorded. The presence of An. arabiensis in such high-altitude areas could be a result of human activities, such as deforestation and/or swamp reclamation, which impact on the micro-climatic conditions of breeding habitats and create environmental conditions that favour mosquito breeding and the invasion of new species.

3. Pictures of the study sites in western Kenya: highland villages of Fort Ternan (a) and Lunyerere (b), and a rice paddy near the peri-urban village Nyalenda (c). Photos: Susan Imbahale

3. Impressie van de onderzoeksgebieden in west Kenia: de op een hoogvlakte gelegen dorpen Fort Ternan (a) en Lunyerere (b), en een rijstveld buiten het nabij de stad Kisumu liggende dorp Nyalenda (c).
Anopheles funestus was recorded mostly as larvae in Lunyerere from habitats that had grass growing in them, whereas in Fort Ternan mostly adult mosquitoes of this species were recorded from houses that were within 500 m of the Kipchorian river; thus, all its breeding habitats were along the river fringe. In Nyalenda, an area where rice was grown, no An. funestus was recorded throughout the study. This is in contrast to other studies, in which An. funestus was commonly found in rice paddies during the late stages of rice development (Klinkenberg et al. 2003). The collective data demonstrated the widespread presence of larval stages of malaria vectors in all study sites and with sufficient ambient temperature for parasite development, malaria will be transmitted locally.

Prevalence of Plasmodium falciparum
The overall monthly mean malaria parasite prevalence in a cohort of school children from Fort Ternan, Lunyerere and Nyalenda was not significantly different among the sites (figure 4) throughout the study. The school children from the highland villages exhibited high prevalence rates when compared to those in the peri-urban Nyalenda. This could have been a result of the low monthly turnout of school children hence contributing to the low prevalence rates recorded. In addition, the residents of peri-urban Nyalenda were more inclined to use mosquito nets, hence protecting themselves from infectious mosquitoes, when compared to the rural population making it much more vulnerable to malaria.

Community involvement in malaria transmission and its control
The majority of mosquito larval habitats recorded were man-made and almost all were linked to agricultural activities. This contributed to the disease burden by providing conducive breeding sites for the malaria mosquitoes. However, a survey done in the current study revealed that many community members did not associate the breeding of mosquitoes with stagnant water or their farming activities (Imbahale et al. 2010). One third of the respondents associated breeding of mosquitoes with bushes and plantations of coffee, sugarcane or maize and consequently many thought that mosquitoes would be controlled through bush clearance. Only few recognized that the major breeding sites are those that they create themselves in their vicinity. If this knowledge is lacking, then we cannot expect that people take any preventive action. It is important to understand that irrigated areas are known to have higher densities of mosquitoes, but this may not automatically lead to higher malaria incidence among the inhabitants, as explained by the paddies paradox (Ijumba & Lindsay 2001). A similar situation was observed in peri-urban Nyalenda, with high densities of adult mosquito vectors collected but where the sporozoite rates and malaria prevalence were relatively low.

Generally, men were more informed concerning the cause of malaria than women, especially in the villages. Women spend considerable time on taking care of sick adults and especially children. In addition, in sub-Saharan Africa, women account for about 70% of agricultural workers and 60%-80% of food crop producers for household consumption and sale (FAO 1996). These findings emphasize the importance of capacity building especially targeting women, to increase knowledge on the causes of malaria and how their activities affect or create opportunities for vector-borne diseases.

Lunyerere provided a good example of land use change leading to disease risk, being a reclaimed natural swamp that is currently under crop cultivation These land use changes have opened up the area, but the continued presence of underground seepage provides small pools of water, favourable to the most efficient malaria vector, An. gambiae, and the invasion of new species like An. arabiensis. Agriculture is the mainstay of rural communities and if these farming communities would understand the link between agriculture and health, this would be one step in solving the problem. Once these links are understood, then suitable control strategies need to be developed in combination with the affected communities. It became clear that, although community members that took part in this study were willing to take part in mosquito vector control, they did not know how they can help (Imbahale et al. 2010). This study has addressed this issue by providing results on a proof of concept that can be adopted in the integrated control of malaria. Integrated mosquito larval control options such as environmental (water) management and biological control can be adopted by farming communities without interrupting the activities on their farm.
Integrated mosquito larval control strategies

The variation in topography and vector species composition in the two highland villages clearly show that intervention strategies are site specific, and all interventions studied are not necessarily suitable for all habitats at all locations. This called for the development of site-specific larval control strategies that can be applied depending on the habitat suitability. Peri-urban Nyalenda is relatively flat, with water present throughout the year for crop and commercial tree nursery irrigation. Because water is needed and consequently it cannot be drained away, application of bio-larvicides such as Bacillus thuringiensis var. israelensis (Bti) with or without combination of larvivorous fish would be feasible. The predatory fish Gambusia affinis (Baird & Girard) was found suitable as it was locally available and it thrived under local conditions in the study areas. The transient nature of temporary habitats required a strategy such as the application of Bti which could be applied weekly as advised by Fillinger & Lindsay (2006).

The effectiveness of Bti for the control of African anophelines has been demonstrated by several studies (Fillinger & Lindsay 2006, Majambere et al. 2007, Geissbühler et al. 2009). The Bti used in this study was imported as it is not locally available; hence, the main limitation is the accessibility of this product to the local population. Apart from the application of Bti, permanent habitats in the rural highland villages of Fort Ternan and Lunyerere were subjected to multiple mosquito larval control options. This was possible due to the distinct differences in topography and the nature of the breeding habitats.

The intervention strategies involved environmental (water) management through source reduction, habitat manipulation by provision of shade and the use of biological control through larvivorous fish (G. affinis) in comparison to the application of Bti. In this case provision of shade was selected for Lunyerere because the main vector species, An. gambiae, prefers to breed in open, sunlit, temporary water pools (Gillies & Coetzee 1987), and hence is not likely to breed in shaded habitats. Conversely, An. funestus thrives in permanent habitats with emergent vegetation. Arrow root plants were not foreseen to provide such a habitat, because as they grow they suck in water, leaving no stagnant water for ovipositing females (figure 5). The arrow root plant produces root tubers that are a good source of carbohydrates. It was envisaged that because it is a source of food for man and can be utilized in controlling mosquito larvae, then the idea would easily be accepted by the farm owners.

One of the remarkable results was that all the intervention strategies applied led to complete elimination of the aquatic stages of both anophelines and non-anopheline species within habitats when compared to the controls. By reducing aquatic stages for both vectors for malaria and nuisance biting mosquitoes, we envisaged that mosquito bites would be reduced or stopped and that the community members will be more willing to take part in vector control activities. In the present study, when the intervention strategies were stopped, mosquito larvae were immediately recorded anew from the respective habitats, implying that for the process to be effective, active management and co-ordination of activities is required at a local level.

Vector control strategies developed with the collaboration of community members, without a financial burden, is envisaged to be sustainable and acceptable in the long term. Source reduction through drainage and provision of shade using locally-grown arrow root crops and biological control using fish were as effective as the application of Bti. Currently, Bti is not accessible by the local community and therefore alternatives such as source reduction, environmental manipulation and use of predatory fish could be adopted. The inhabitants of the respective communities were aware of the fact that source reduction
(drainage of stagnant water) would be useful for mosquito control; however, it was not clear how many practiced it (Imbahale et al. 2010). The control of aquatic stages of mosquitoes can be incorporated into the current integrated malaria control programmes that mainly target adult mosquitoes and treatment of malaria parasites. Although spatially limited, this study provides evidence-based combinations of mosquito larval control strategies which can be explored on a large scale.

Addressing the malaria-agricultural interface requires a broad interdisciplinary and integrated approach that involves local communities and more than one public sector (van den Berg & Knols 2006). There is a need to build bridges between sectors that currently tend to work in isolation (Singer & de Castro 2007).

Conclusion
Sustainable disease control programs need to involve the concerned communities in their implementation. The community perceptions, current knowledge on the disease malaria and their cultural values with regard to its cause and control need to be considered. This study has shown that mosquito larval control through environmental management and biological control utilizing locally available options compared well with the application of Bti. However, for this to be possible there is a need for active management with frequent monitoring and evaluation, which can be achieved with institutional support and village volunteers. Future research needs to test the strategies developed on a large scale, with active involvement of communities and to develop other novel strategies that are applicable to transient mosquito habitats. However, for this to be practical there is a need for collaboration between the different institutions involved in malaria control so that a lasting solution can be developed.

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**Samenvatting**

Geïntegreerde bestrijding van malariavectoren in verscheidene landbouw-ecosystemen in West-Kenia

Malaria is een complexe ziekte, waarvan de mate van ernst bepaald wordt door de wisselwerking tussen de vector (een mug van het geslacht *Anopheles*), de parasiet (een protozo van het geslacht *Plasmodium*), de gastheren en de omgeving. Bestrijding van malaria is vooral gericht geweest op de parasiet en op de volwassen muggen. Maar de ontwikkeling van resistentie bij parasieten en muggen maken de ontwikkeling van alternatieve strategieën noodzakelijk, zoals de bestrijding van muggenlarven, die kan worden geïntegreerd in bestaande bestrijdingsprogramma’s. Om een larven-bestrijdingsprogramma te ontwikkelen, dienen de plaatselijke vectorsoorten en hun wijze van voortplanting bekend te zijn. Dit proefschrift beschrijft de resultaten van onderzoek naar de ecologie van muggen, meer in het bijzonder onderzoek aan malariavectoren in verscheidene landbouwgebieden in West-Kenia, te weten twee op een hoogvlakte gelegen gebieden (Fort Ternan en Lunyerere) en een gebied in een meer stedelijke omgeving (Nyalenda). Daarnaast beschrijft dit proefschrift de ontwikkeling van praktische en effectieve bestrijdingswijzen van muggenlarven. Om meer omtrent de ecologie van de plaatselijke vectoren te weten te komen, is in de drie genoemde gebieden gedurende twee jaar een doorlopend onderzoek uitgevoerd naar enerzijds het vóórkomen van malaria en anderzijds de populatiedynamiek en voorplantingskarakteristieken van de betreffende muggensoorten. Daarnaast is door middel van enquêtes onderzoek gedaan onder de plaatselijke bevolking naar de kennis van en het omgaan met malaria, zijn oorzaak en zijn bestrijding. Met al deze informatie konden we kleinschalige larvenbestrijdingsplannen ontwikkelen, op basis van bronverkleining, manipulatie van de omgeving door meer schaduwplekken, en biologische bestrijding met behulp van roofvissen (*Gambusia affinis*) en een bio-larvicide, *Bacillus thuringiensis var. israelensis* (*Bti*). De belangrijkste malaria-vectorsoorten, *Anopheles gambiae* Giles sensu stricto en *An. arabiensis* Patton, waren allebei als larve te vinden op alledrie de plekken, terwijl *An. funestus* Giles alleen gevonden werd bij de twee hooggelegen dorpen. De meeste (86%) van de muggenbroedplaatsen bleken te zijn veroorzaakt door menselijke activiteit. De belangrijkste malariaparasiet in de omgeving, *Plasmodium falciparum*, werd aangetroffen in de onderzochte groepen schoolkinderen en de aanwezigheid van malaria bleek niet erg te verschillen tussen de drie gebieden. De bewoners van de drie gebieden beschouwden malaria als een last en ze gaven aan bereid te zijn mee te willen werken aan de bestrijding van muggen, al wisten ze niet hoe dat te doen. Een proef (‘pilot’) in het veld toonde de haalbaarheid en de effectiviteit aan van larvenbestrijding in door de mens gemaakte (landbouw) muggenhabitats. Een geïntegreerde benadering met behulp van plaatselijk voorhanden larvenbestrijdingsmethoden kan gemakkelijk worden opgepakt door de betreffende bevolking. Betrokkenheid van de bevolking (‘community involvement’) bij de bestrijding van ziekten zal leiden tot inzicht in het effect van menselijk handelen op de eigen gezondheid en op het vóórkomen en voorkómen van ziekte.

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