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Can repellents prevent malaria in Tanzania?

Peter Onyango Sangoro

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Department of Disease Control

Faculty of Infectious and Tropical Diseases

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Declaration

This work was supervised by:

Dr. Mary Cameron, PhD,

Senior Lecturer

Department of Disease Control, Faculty of Infectious and Tropical
Diseases, London School of Hygiene and Tropical Medicine

I, Sangoro Peter Onyango, confirm that the work presented in this thesis is my own and has not been submitted for another degree or diploma at any university or institution of tertiary education. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. The contributions made by other authors in this work have also been acknowledged.

Signature: 

Date: 10th August 2015

Abstract

Background: Current malaria control tools, long lasting insecticidal nets and indoor residual spraying have had a significant impact on malaria transmission in sub-Saharan Africa. However these tools will not be able to eradicate malaria and there is need for complementary tools if this goal is to be attained. This work focused on evaluating and recommending tools that can be used to complement current control tools with emphasis on outdoor and early evening transmission. The main tool evaluated in this thesis was a topical repellent to be used in the early evening. Other tools recommended were spatial repellents and permethrin-impregnated clothing.

Methods: A repellent efficacy trial was conducted in the semi-field and field setting to evaluate the protection from early evening biting given by a topical repellent lotion containing 15% *N,N*-diethyl-*m*-toluamide (DEET). A cluster randomized, placebo controlled clinical trial, designed to assess the effect of 15% DEET against malaria transmitted in the early evening was then conducted in a village in rural Tanzania. A total of 940 households were recruited and randomized, with 462 households randomized to the intervention arm and 462 households randomized to the control arm. The feasibility of lotion repellent use was assessed using entry and exit questionnaires. Focus group discussions were conducted 3 years after a clinical trial to assess the community knowledge, attitude and practice towards a different set of repellents to those used during the clinical trial. A systematic review was then conducted to put the results of this trial in context with other repellent trials. An attempt was made to design a clinical trial taking into account the shortcomings of the current and other repellent trials reviewed.

Findings: Topical repellents containing 15% DEET provided >80% protection against early evening biting over four hours. According to protocol analysis of the cluster randomized trial found no difference between the intervention and control arms after accounting for socio-economic status, education of household head and household construction materials (Wilcoxon rank sum $z = 0.529$, $p = 0.596$). The most important predictor of malaria in this study was age, with younger age categories significantly associated with greater malaria risk. Socio-economic status was not associated with malaria. Compliance to repellent use was reported to be 80% during the study. From the FGDs, it emerged that community knowledge was the major barrier to repellent use, followed closely with availability. The community preferred using long lasting insecticidal nets (LLINs) because of their cost effectiveness. However, the community preferred using repellents in the early evening before employing LLINs.

Interpretation: This study demonstrates that topical repellents have no effect against early evening malaria transmission in this community. However, shortcomings in the design and implementation might have masked the treatment effect and better-designed studies are required to establish repellents effect in this setting. Topical repellents provided protection against early evening biting and were readily accepted and used in this community, indicating the potential of using repellents complimentary to LLINs in this setting. The short-term duration of effect of this repellent, required frequent reapplication and therefore impacted compliance, emphasizing that future studies should consider using longer lasting tools such as spatial repellents.

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And to *Ba*, I hope you are smiling down old man.

Dedication

For Imani, Neema and Kweli.

You can achieve anything by simply applying yourself.

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Acronyms

ACT	Artemisinin-based combination therapy
ATP	According to protocol
CI	Confidence Interval
CPT	Complete protection time: The time between application of a repellent and first mosquito landing
DDT	Dichlorophenyltrichloroethane
DEET	<i>N, N</i> -Diethyl-3-methylbenzamide
ED50	Effective dose required to protect from 50% of mosquitoes
ED99.9	Effective dose required to protect from 99.9% of mosquitoes
GLM	Generalized linear models
HBI	Human blood index
HLC	Human landing catch
IHI	Ifakara Health Institute
IRS	Indoor residual spraying
ITNs	Insecticide treated nets
IVM	Integrated vector management
IQR	Interquartile range
ITT	Intention to treat
LLIN	Long lasting insecticidal net
LSHTM	London School of Hygiene & Tropical Medicine
NOEL	No observable effect level
MOE	Margin of Exposure
OR	Olfactory receptors
PCR	Polymerase chain reaction
RDT	Rapid diagnostic test
SR	Spatial repellents
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme

ABSTRACT

Background: Currently, the major malaria vector control tools, long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) predominantly target vectors biting and resting indoors. Therefore, despite the impact of these tools on malaria transmission as a result of their extensive use, there is still some substantial transmission taking place outdoors and in the early evenings, when and where their impact is diminished. Consequently, current malaria control tools will not be able to eliminate malaria and there is need to develop new tools and strategies targeting this residual transmission if this goal is to be achieved

Objectives and Methods: The main objective of this PhD is to evaluate the additional benefit of using 15% DEET topical repellent to LLINs in preventing malaria transmission in the early evening, assessed through a cluster randomized, placebo-controlled clinical trial in a rural village in Tanzania. The efficacy of 15% DEET topical repellent was assessed by conducting human landing catches in the semi field system (SFS) as well as the field against laboratory reared *Anopheles arabiensis* and wild mosquitoes respectively. The acceptability of 15% DEET topical repellent as a mosquito control tool was assessed using before and after studies and focus group discussions (FGDs). A literature review on the impact of repellents on disease transmission was also conducted to align the results of this work in context with findings from other trials.

Results and conclusions: Analysis of the randomized clinical trial demonstrated a non-significant 11.4% reduction in malaria incidence when the repellent arm was compared to the placebo arm (Wilcoxon rank sum $z = 0.529$, $p = 0.596$). It was therefore deduced that 15% DEET topical repellent did not have any impact on malaria transmission in the early evening. However, 15% DEET topical repellents

provided >80% protection against laboratory reared *An. arabiensis* and wild mosquito bites in the SFS and field setting respectively. The repellent was also readily acceptable, with >99% of the community reporting willingness to use the repellent. The reported monthly compliance was 79%. Cost and accessibility/availability were found to be the main barriers to use of repellents in rural Tanzania. Results from this trial of no effect of 15% DEET on malaria transmission was consistent with a study conducted in Lao-PDR using exactly the same repellent. However these findings were inconsistent with studies carried out in Bolivia, Pakistan, Ethiopia and Ghana.

Interpretation: The results demonstrated that topical repellents did not have additional benefit to LLINs in preventing early evening malaria transmission in rural Tanzania. However, there are several factors that might have confounded the findings of this trial. First, the trial lacked any statistical power to observe any discriminatory difference between the two treatment groups. Secondly, compliance to repellent use could not be effectively established. There was also an imbalance in socio-economic status between the treatment arms. During the study period there was low malaria transmission as a result of a drought in Tanzania. However, repellent use was readily accepted in the community and therefore has the potential to be used as a control tool against early evening mosquito biting.

This thesis outlines the shortcomings of the study design of this trial and gives recommendations on how they can be addressed in future trials. A detailed outline of a protocol on how a study using similar interventions (repellents – spatial or topical) should be conducted has been included in this body of work in an attempt to standardize the design and implementation of future trials so that outcomes are considered robust and of good quality. I believe that this information will be critical in

designing future trials using similar interventions and will add to the body of knowledge of repellents that already exists.

Chapter 1: General Introduction

1.1 Malaria epidemiology in Africa

There are several factors that propagate malaria transmission in sub-Saharan Africa. First, the climate in sub-Saharan Africa offers ambient optimal conditions for the *Anopheles* sub-genus mosquito to thrive by offering adequate rainfall for proliferation of breeding sites around human populations and ambient temperatures and humid conditions to prevent desiccation of mosquitoes during blood meal foraging [1, 2]. As a result mosquitoes belonging to the *Anopheles gambiae* complex and the *Anopheles funestus* group continue to be the most widely distributed malaria vectors in sub-Saharan Africa (Figure 1:1) and are the major vectors transmitting malaria in this region [3]. The *An. gambiae* complex is made up of 6 sibling species, *An. gambiae* sensu stricto. , *An. arabiensis*, *An. quadriannulatus A*, *An quadriannulatus B*, *An. melas*, *An. merus* and *An. bwambae* [4]. The *Anopheles funestus* group is made up of nine sibling species: *An. funestus* s.s., *Anopheles rivulorum* Leeson, *An. leesoni* Evans, *Anopheles vaneedeni* Gillies & Coetzee, *An. parensis* Gillies, *An. confusus* Evans & Leeson, *An. aruni* Sobti, *An. fuscivenosus* Leeson, and *An. brucei* Service, which are all difficult to distinguish morphologically at the adult stage [4]. All members of the *Anopheles* sub-genus are capable of being infected with the *plasmodia* parasite, and hence can potentially transmit malaria [4, 5]. The major malaria transmitting vectors in Africa are *An. gambiae* s.s. and *An. arabiensis* (*An. gambiae* s.l.) from the *An. gambiae* complex and *An. funestus* s.s. from the *An. funestus* group [4, 5]. *An. gambiae* s.s. and *An. funestus* are known to be predominantly anthropophilic (prefer to feed on humans) [4, 5], endophilic (prefer to feed indoors) [4, 6], endophilic (prefer to rest indoors) [4] and bite late at night [4].

An. arabiensis, thought to be previously zoophagic (prefer to feed on bovines) has been shown to exhibit an elastic biting behaviour, feeding either on cattle [6] or humans depending on the available hosts [4, 5], resting indoors [6] in the absence of outdoor resting sites or outdoors if resting sites are available [6, 7]. *An. arabiensis* has also been shown to feed either indoors [8] or outdoors [6]. *An. gambiae* s.l. (*An. gambiae* s.s and *An. arabiensis*), predominantly breeds in transient aquatic habitats like hoof prints, car ruts and in rice paddies (man-made habitats) [4]. As a result, these vectors are found in great densities and close proximity to humans [4, 5, 9]. On the other hand, *An. funestus* breeds in large, clear and semi-permanent water bodies like swamps, rice paddies and ponds. Owing to its anthropophilic, endophilic and endophilic behaviour [6, 8, 10], this vector colonizes habitats that are close to human dwellings, like rice paddies [10].

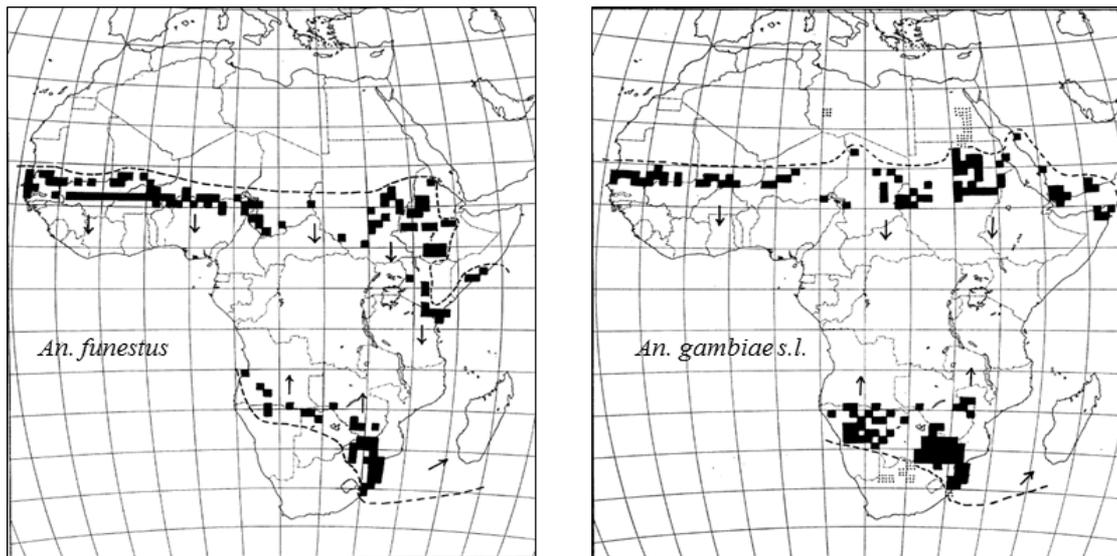


Figure 1:1 Map of Africa showing distribution of *An. funestus* and *An. gambiae s.l.* species complex mosquitoes. Source [4, 11]

All the above parameters; extensive distribution of these vectors, close proximity to man due to man-made breeding sites and high survival rates as a result of ambient conditions, combine to create optimal conditions for transmission of malaria parasites by *An. gambiae s.l.* and *An. funestus* vectors, and is known as vectorial capacity [2, 3]. Vectorial capacity (VC) is described as the number of secondary cases that arise from one infective case per day in a susceptible human population and is dependent on; ratio of mosquito populations to humans (m), the human biting rate per day (a), survival rate of the mosquito per day (p) and the length of the gonotrophic cycle (n):

$$C = \frac{ma^2pn}{- \log_e p}$$

$$- \log_e p$$

Secondly, mosquitoes belonging to the *Anopheles* sub-genus are the only mosquitoes known to transmit to *Plasmodium falciparum* [4], the most virulent form of the

parasite that causes malaria and is predominantly found in sub-Saharan Africa [1, 12, 13].

Then there is the problem of underdeveloped health systems and poor infrastructure in most sub-Saharan African countries to drive any substantial campaign against malaria [14].

All these factors: the most competent malaria vector, coupled with the most virulent malaria parasite pitted against a poor and under developed infrastructure and health system, combine to create an efficient malaria transmission system that requires considerable financial and political resources and commitment to have any significant impact on transmission.

1.2 Change in Malaria epidemiology in sub Saharan Africa

The last two decades has seen substantial financial and political commitment employed in malaria control. The funding committed to malaria control was estimated to be US \$ 2.5 billion in 2012, up from US \$ 100 million committed in 2000 [15].

This increase in funding has led to extensive scaling up vector control tools like LLINs and IRS as well as cost-effective diagnosis kits, (rapid diagnostic tests – RDTs) and Artemisinin-based combination therapy (ACT) drugs for prompt diagnosis and treatment of malaria. Targeted prevention of malaria among the groups at risk, such as pregnant mothers and infants using intermittent preventive treatment (IPTp and IPTi) is also being extensively used [15]. As a result of scale up of malaria control tools, several regions of malaria endemic sub-Saharan Africa have observed a decline in malaria related morbidity and mortality.

In the Horn of Arica, surveys carried out in Ethiopia demonstrated a decline in the prevalence of malaria morbidity [16] and under five morbidity and mortality [17]. In Eritrea LLINs, IRS and larviciding were associated with decline in malaria cases from

1998 to 2003 [18] while a retrospective study using HMIS data to assess the impact of ITNs and IRS and a longitudinal study assessing the impact of LLIN scale up both demonstrated declining trends in malaria morbidity [19, 20] and mortality [20].

However, it should be noted that malaria prevalence was already declining before the scale up of mosquito control tools and the decline might have been associated with climatic factors [18, 21] alongside scale up of malaria control tools.

In East Africa, studies exploring malaria trends have reported declines in malaria morbidity and mortality along the Kenya coast after 18 years of surveillance [22], and in outpatient malaria cases in Central Kenya after long term implementation of integrated malaria management (IMM) [23]. A country wide survey assessing the trend of malaria related hospital admissions in 17 district hospitals reported an average overall decline of 49% from 1999 to 2008 [24], although this temporal trend was not observed in all hospitals indicating that there were different factors affecting transmission in the different hospital settings [21, 25]. Declines in malaria trends have also been reported from highland areas in Kenya indicating that malaria is declining even in areas of unstable transmission further reinforcing the finding of declining malaria transmission in sub-Saharan Africa. Declines in malaria trends associated with scaling up of malaria control tools in other parts of East Africa have also been reported in Burundi [26] and Tanzania [27].

In Southern Africa, integrated malaria control by use of IRS and ACT (Artemether-Lumefantrine), in Kwa Zulu Natal in South Africa was associated with declines in malaria morbidity and mortality [28], while in Swaziland use of IRS was associated with declines in malaria related morbidity [29]. Concurrent use of LLINs, IRS, ACT and RDTs was also associated with a decline in malaria morbidity in Zambia [30].

In West Africa, declines in malaria trends associated with scaling up of control tools have been reported in The Gambia [31] and Gabon [32].

Declines in malaria trends have also been observed on the African Islands of Zanzibar [33], Bioko Islands in Equatorial Guinea [34] and Sao Tome and Principe [35].

In addition to having reduced the burden of malaria in sub-Saharan Africa, scaling up of malaria control tools have also had an impact on entomological parameters relating to malaria transmission, such as vectors density, parasite prevalence and entomological inoculation rate (EIR). Extensive use of LLINs has been associated with declines in EIR and vector densities in Tanzania [36-38], Kenya [24, 39, 40], Solomon Islands [41] and Nigeria, while IRS has been shown to reduce EIR in Tanzania [37], Solomon Islands [41] and Nigeria [42]. Even the Kilombero valley, historically one of the places with the highest malaria transmission in the world, has reported up to a 4-fold decline in EIR [43]. In addition to their impact on EIR, malaria control tools have also been shown to reduce parasite prevalence [44-46].

However, not all areas where malaria control tools have been implemented have demonstrated a decline in malaria trends [19, 20, 24], indicating that malaria transmission is influenced by a variety of factors [21, 25].

Urbanization, rapidly taking place in sub-Saharan Africa, has also affected malaria transmission and a negative linear relationship has been observed between EIR and the level of urbanization [47, 48]. This is mainly a result of the impact of urbanization on larval habitats, access to better health facilities, awareness of protective methods against mosquito bites, and more effective larval control as breeding sites are well defined and therefore easier to manage [48].

Long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) predominantly employ the use of pyrethroid insecticides. As a result, scale up of these

tools for universal coverage has led to extensive use of pyrethroids. Inevitably, malaria vectors have developed resistance to pyrethroids, thereby influencing further the transmission dynamics in sub-Saharan Africa [49], so that areas where malaria transmission had once declined are reporting disease resurgence [34, 50, 51] and the re-emergence of previously eliminated malaria vectors [11]. However, there are areas where LLINs and IRS continue to demonstrate effectiveness despite high frequencies of pyrethroid resistant alleles being observed in the local malaria vector population [26, 52, 53] reinforcing the fact that more research is needed in understanding mechanisms of resistance development in malaria vectors [49].

Lastly, extensive use of LLINs and IRS has been implicated in modifying mosquito behaviour and species composition. Apart from reducing the indoor resting densities of mosquitoes as a result of mosquito control tool-induced mortality [54, 55], these tools have also been reported to shift the peak biting times of malaria vectors [56-58], feeding preference from either endophagy to exophagy or vice versa [42, 58-61], and resting preference from either endophily to exophily or vice versa [62-64]. Reduction, up to elimination of species of the local predominant vector has also been observed and associated with scale up of control tools, leading to colonization of a once predominant species by alternative vector species [65-67]. Larval sampling in places where there has been extensive LLIN and IRS employment also demonstrate a shift in the sub-populations of the local malaria vectors [63, 64].

All these factors above have had a great impact on malaria transmission in sub-Saharan Africa and as a result, most of sub-Saharan Africa is continually evolving from a region of high endemicity with homogenous transmission to a region of moderate to low endemicity with heterogeneous transmission, characterized with transmission hotspots [68, 69]. Consequently, further scale up of current control tools

will not be able to have any additional impact on these localized foci of residual transmission [68, 70]. Therefore new tools and strategies targeting these residual transmission foci will need to be developed if malaria is to move from sustained control to elimination. One area of residual transmission focus that presents a challenge for control is outdoor and early evening malaria transmission[70, 71]. As such, the main theme of this thesis was therefore to explore supplementary tools that could be used to address outdoor and early evening transmission with emphasis on using repellents as a means of personal protection outdoors and in the early evening.

1.3 Statement of the problem

Current vector control tools, LLINs and IRS predominantly target intra-domiciliary malaria vectors. However, as a result of extensive use [15], these tools are increasingly being implicated in shifting malaria vector species composition, so that vector populations of indoor biting and resting vectors are being diminished, while populations of outdoor biting and resting vectors become dominant [71]. This shift in behaviour presents a challenge for malaria control, as LLINs and IRS are unlikely to impact on the transmission mediated by these outdoor biting and resting vectors.

Other factors that are likely to attenuate the impact of LLINs and IRS is the development of insecticide resistance [49]. As a result, despite reduced transmission, malaria is unlikely to be eliminated in such a scenario with continuous implementation of these tools (LLINs and IRS) and a new strategy needs to be developed. To tackle increasing insecticide resistance the WHO recommends development of new insecticides and rotation of current ones where cross-resistance has not developed [49]. For outdoor transmission, there is need to develop novel tools or strategies to tackle this residual transmission foci. In the context of this work,

outdoor and early evening transmission will be defined as a transmission focus and will form the basis of this PhD thesis.

One potential strategy is the use of topical repellents outdoors and in the early evening before employment of LLINs and IRS. Repellents have been previously described as chemicals that induces oriented vector movement away from the chemical source [72]. However, repellency in the context of this work is described broadly as a range of insect behaviours induced by airborne chemicals that result in a reduction in human-vector contact and therefore offering personal protection and includes movement away from the repellent, interference with host detection as well as feeding inhibition [73]. Repellents can be either be topical (chemicals applied on the skin that prevent vector-host contact) [72] or spatial (chemicals diffused in the air to prevent vector-host contact in a defined space) [74]. Repellents are classified as either natural (plant-based) or synthetic [75]. Most plant-based repellents are derived from pyrethrum (*Chrysanthemum cinerifolium*) flowers and can either be used to knock down or repel disease vectors. Other plant-based repellents include lemon eucalyptus (derived from *Eucalyptus maculata citriodon*), citronella (*Cymbopogon* genus) and neem (*Azadirachta indica*). However, most plant based repellent have a short residual effect [76]. The need for longer lasting repellents therefore led to development of synthetic repellents.

N, N-diethyl-3-methylbenzamide (DEET), the most widely used synthetic repellent (over 200 million annual applications) to date, was developed in the 1950's by the United States Department of Agriculture (USDA). Currently, a number of synthetic repellents exist, but DEET is considered the gold standard topical repellent, because it is broad-spectrum (efficacy against a variety of arthropods) as well as long lasting. This work particularly focused on the efficacy of DEET topical repellent on malaria

transmission in the early evening.

1.4 Safety and toxicity of DEET

N, N-Diethyl-3-methylbenzamide (DEET) was used in this study because it has been extensively tested for safety and toxicity for human use [77-79] and its efficacy against a broad variety of arthropod vectors [76, 80, 81]. DEET was first registered in 1957. However reports of DEET related side effect prompted reviews and investigations into DEET safety. It was found that for over 50 years since DEET was discovered there were only 43 case reports of DEET toxicity to the Poisons and Control Centers in the US [76], of which 26 severe cases occurred between 1995-1997 [82] and 14 from 1985-1989 [79] (Tables 1:1 & 1:2). Of all these reported cases, 5 patients died, of which the deaths were due to incorrect use of DEET like ingestion and inhalation, while the rest resolved without any sequelae [83]. There have also been concerns on effect of DEET on children, pregnant and lactating mothers. However recent studies have conclusively demonstrated that DEET is safe for use by these groups [84-86].

Despite its long-term and extensive use, the exact mode of action of action of DEET is yet to be established. Existing theories have suggested the following modes of action: Inhibiting response to an otherwise attractive host signal [87, 88], switching the sensory message from attraction to repulsion [89], activating a receptor system that controls a competing behaviour [89], activating a noxious odour receptor [90, 91] and activating different receptor types simultaneously causing loss of the specific signal for host finding [89].

Table 1:1 Summary of reported cases with severe outcomes or death that occurred from 1986-1989. Source: [104, 106]

Effects	No. of patients	Age (sex)	Deet level (%)	Symptoms	Reference
Death	1 ^a	17 months (female)	20	Acute encephalopathy	[92]
Death	1 ^a	6 years (female)	15	Abdominal pain, vomiting, lethargy, headache, ataxia, general convulsions	[93]
Death	1 ^a	6 years (female)	10	Headache, agitation, atethosis, disorientation, involuntary movements and convulsions	[94]
Encephalopathy	1 ^b	5 years (female)	95	Convulsions	[95]
Encephalopathy	1 ^b	8 years (male)	Unspecified	Convulsions	[78]
Encephalopathy	5 ^b	3-7 and 29 years (male)	Unspecified	Seizures, one developed urticaria	[96]
Encephalopathy	1 ^b	18.5 months (female)	20	Tremors, progressive ataxia and weakness, bizarre movements	[97]
Encephalopathy	1 ^b	8 years (female)	100	Seizures, convulsions, erythematous and pruritic rash, unusual restlessness	[98]
Encephalopathy	1 ^b	3.5 years (female)	15	Convulsions, incoherent speech, stiff arms and legs	[99]
Acute manic psychosis	1 ^b	30 years	Unspecified	Psychomotor hyperactivity, rapid and pressured speech, tangentiality, flight with ideas and grandiose delusions	[100]
Cardiovascular toxicity	1 ^b	61 years (female)	Unspecified	Light-headedness, nausea, vomiting, explosive diarrhoea, hypotension	[101]
Anaphylaxis	1 ^b	42 years (female)	52	Generalized angioedema, nausea, loss	[102]
Reproductive toxicity	1 ^b	4 years (male)	Unspecified	Mental retardation, impaired sensory-motor coordination, cranio-facial dysmorphism	[103]
Encephalopathy	1 ^b	17 months (female)	20	Opisthotonos, respiratory difficulty, coma	[92]
Seizures	1 ^b	1.5 years (Male)	10	Opisthotonos, respiratory difficulty, coma	[104]
Encephalitis	1 ^b	1.5 years (female)	10	Agitation, opisthotnos	[94]
None	1 ^b	14 (female)	95	Coma, hypotension, hypertonia	[105]
None	1 ^b	1 (female)	47.5	Coma, seizures, hypertonia, opisthonos	[105]
None	1 ^b	16 (female)	95	Coma	[105]

Table 1:2 Summary of the reported cases with severe outcomes or death that occurred from 1993-1997. Source: [107]

Effect	No. of patients	Age category	Deet level	Symptoms	Reference
Death	1 a	Adult (female)	>50%	Dermal	[107]
Death	1 a	Adult (male)	>50%	Gastrointestinal, neurological, cardiovascular, respiratory	[107]
	1 b	Infant (male)	Unknown	Neurological	[107]
	1 b	Infant (male)	Unknown	Respiratory	[107]
	1 b	Infant (female)	Unknown	Neurological	[107]
	1 b	Child (male)	Unknown	Ocular	[107]
	1 b	Child (male)	Unknown	Unknown	[107]
	1 b	Child (male)	11-50%	Neurological	[107]
	1 b	Child (male)	<11%	Unknown	[107]
	1 b	Child (male)	Unknown	Unknown	[107]
	1 b	Child (male)	11-50%	Ocular	[107]
	1 b	Child (female)	11-50%	Ocular	[107]
	1 b	Teen (male)	Unknown	Gastro-intestinal, hematologic/hepatic, neurological	[107]
	1 b	Teen (male)	11-50%	Neurological	[107]
	1 b	Teen (female)	11-50%	Neurological, miscellaneous other	[107]
	1 b	Adult (male)	Unknown	Neurological	[107]
	1 b	Adult (male)	11-50%	Unknown	[107]
	1 b	Adult (male)	11-50%	Cardiovascular, neurological, respiratory,	[107]
	1 b	Adult (male)	11-50%	Unknown	[107]

1 b	Adult (male)	11-50%	Unknown	[107]
1 b	Adult (female)	Unknown	Unknown	[107]

However, before any topical repellent can be recommended for use, their efficacy has to be tested and established. There are several ways in which repellents can be tested. The efficacy of repellents can be tested in the laboratory to determine its complete protection time (CPT): the time between repellent application and first mosquito landing or effective dose (ED): the concentration of repellent required to protect against a given proportion of mosquito bites. Laboratory tests are usually conducted as per guidelines provided by the WHO Pesticide Evaluation Scheme (WHOPES), [108]. Several laboratory experiments have shown DEET to be efficacious against a wide range of arthropods [80, 109-113].

The efficacy of repellents can also be estimated in the semi field and field against laboratory reared and wild mosquito species respectively [114], usually assessed by determining the reduction in proportion of mosquitoes biting a volunteer using a repellent (DEET) relative to the control [114-116].

Apart from assessing the efficacy against mosquito bites, repellent efficacy against disease transmission should be determined. The most credible method of assessing repellent efficacy against disease transmission is through randomized controlled trials where one group of participants are given the repellent being tested, while another group with similar baseline characteristics are given a placebo or no intervention and the proportion of disease between these two groups is determined.

There are several randomized controlled trials that have reported efficacy of topical repellents against malaria transmission: A household-randomized, double blind, placebo-controlled clinical trial was conducted in Bolivia. All participants of the study were given ITNs so as to standardize the malaria interventions used among the participants. The participants in the intervention arm were then issued with a repellent lotion containing 30% p- methane-3-8-diol (PMD), a repellent derived from lemon eucalyptus). In the control group, participant were issued with 0.1% clove oil. PMD was associated with an 80% reduction in *Plasmodium vivax*

malaria and a non-significant reduction of 82% in *P. falciparum* malaria [117]. In a refugee settlement in Pakistan, a household randomized trial of Mosbar (a soap containing 20% DEET and 0.5% permethrin) compared to a placebo lotion demonstrated a statistically significant 56% reduction in *Plasmodium falciparum* malaria [118]. In another refugee camp in Thailand, a double-blind randomized clinical trial evaluating the effect of DEET mixed with *thanaka* (a root paste made from pulp of the wood of apple tree) compared to *thanaka* alone in pregnant women, demonstrated a 28% reduction in malaria incidence in women who used *thanaka* and DEET, compared to the ones who used *thanaka* alone [84]. In addition to randomized trials, a case control study of Mosbar, similar to the one used in the Pakistan refugee camp was conducted in Afghanistan. Use of Mosbar was associated with a 92% reduction in the odds of contracting malaria [119].

In Africa, the use of repellents as malaria control tools has been scanty and only recently has the efficacy of topical repellents on malaria transmission been evaluated. A cluster randomized controlled trial was conducted in Ethiopia to determine the effect of Buzz Off repellent on malaria. The use of Buzz Off repellent was associated with a 43% reduction in the odds of contracting malaria [120]. In a community-wide study conducted in Ghana to determine the effect of NO-MAS mosquito repellent on malaria prevalence, use of NO-MAS was associated with a 60% reduction in malaria prevalence in the repellent village relative to the control village where no repellent was issued [121].

In line with achieving the millennium development goal (MDG) of reversing malaria by 75%, Tanzania has rapidly scaled up use of LLINs to all at risk groups [122]. While this is likely to have a significant impact on malaria transmission, it might also lead to a situation where vectors adapt to feeding at times and places when and where LLINs are not employed. As a result, new tools will be needed to impact on this transmission foci where LLINs are not effective. Therefore, and following from the findings of other repellent trials above, the main

objective of this work was to assess the impact of using 15% DEET topical repellent on early evening malaria transmission in a rural village in Tanzania. However, as the efficacy of topical repellents is highly dependent on daily compliance which is likely to impact on the uptake of topical repellents and therewith its efficacy, a feasibility study of topical repellent uptake was also conducted using before and after studies and focus group discussions (FGDs). Further, the shortcomings of this work are also outlined and recommendations on how to conduct future trials given.

1.5 Hypothesis

To test the hypothesis that use of 15% DEET topical repellent in conjunction with LLINs significantly prevents malaria transmission in the early evening in rural Tanzania, compared to the null hypothesis that use of 15% DEET topical repellents with LLINs has no significant impact on malaria transmission in the early evening, [84, 117-121], a cluster-randomized, placebo controlled clinical trial was conducted in a rural village in Tanzania. The efficacy of 15% DEET topical repellent against mosquito bites was also assessed in the semi-field and field against laboratory-reared and wild *An. arabiensis* mosquitoes respectively. The feasibility of using topical repellents as a malaria control tool in rural Tanzania was assessed through a Knowledge attitude and practice (KAP) survey.

1.6 Objectives

The overall aim of this study was to determine whether topical repellents are a suitable intervention against malaria in rural Tanzania. The specific objectives are:

1. To determine the efficacy of 15% formulated DEET topical repellent under semi-field and field conditions against laboratory-reared and wild *An. arabiensis* mosquito populations respectively. This work made up in chapter 2 of this thesis
2. To evaluate the incremental benefit of using 15% formulated DEET topical repellents in addition to LLINs against RDT-confirmed malaria transmission in the early evening as compared to areas of exclusive LLIN use. This work made up chapter 3 of this thesis
3. To assess the community uptake and acceptance of topical repellents as a protective method against mosquito bites and therefore determine its feasibility as an intervention tool against malaria in Tanzania. This work made up chapter 4 of this thesis.
4. To perform an in-depth literature review, on existing studies on the impact of repellents against disease transmission, with a focus on malaria. This work made up chapter 5 of this thesis.
5. To develop a protocol outlining the design and implementation of a study evaluating the impact of permethrin-impregnated clothing on outdoor malaria transmission. This work made up chapter 7 of this thesis.

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Chapter 2: Use of a semi-field system to evaluate the efficacy of topical repellents under user conditions provides a disease exposure free technique comparable with field data



2.1 Abstract

Background

Before topical repellents can be employed as interventions against arthropod bites, their efficacy must be established. Currently, laboratory or field tests, using human volunteers, are the main methods used for assessing the efficacy of topical repellents. However, laboratory tests are not representative of real life conditions under which repellents are used and field-testing potentially exposes human volunteers to disease. There is, therefore, a need to develop methods to test efficacy of repellents under real life conditions while minimizing volunteer exposure to disease.

Methods

A lotion-based, 15% *N, N*-Diethyl-3-methylbenzamide (DEET) repellent and 15% DEET in ethanol were compared to a placebo lotion in a 200 sq m (10 m × 20 m) semi-field system (SFS) against laboratory-reared *Anopheles arabiensis* mosquitoes and in full field settings against wild malaria vectors and nuisance-biting mosquitoes. The average percentage protection against biting mosquitoes over four hours in the SFS and field setting was determined. A Poisson regression model was then used to determine relative risk of being bitten when wearing either of these repellents compared to the placebo.

Results

Average percentage protection of the lotion-based 15% DEET repellent after four hours of mosquito collection was 82.13% (95% CI 75.94-88.82) in the semi-field experiments and 85.10% (95% CI 78.97-91.70) in the field experiments. Average percentage protection of 15% DEET in ethanol after four hours was 71.29% (CI 61.77-82.28) in the semi-field system and 88.24% (84.45-92.20) in the field.

Conclusions

Semi-field evaluation results were comparable to full-field evaluations, indicating that such systems could be satisfactorily used in measuring efficacy of topically applied mosquito repellents, thereby avoiding risks of exposure to mosquito-borne pathogens, associated with field-testing.

Keywords

Repellent, *Anopheles arabiensis*, Semi-field system, Efficacy, *N, N*-diethyl-3-methylbenzamide (DEET)

2.2 Background

Evaluations of topical repellent efficacy against blood feeding arthropods require standardized laboratory and field tests [1-3]. However, conditions in the laboratories are not representative of real life settings where repellents are used. Therefore, experiments carried out in the laboratory may not accurately estimate the efficacy of repellents in the field [4]. Environmental factors such as temperature, humidity and wind speed, all of which affect the effectiveness of repellents, are controlled in the laboratory, but in the field these factors may fluctuate and affect repellent efficacy [5]. As a result, tests carried out in the laboratory ideally should be verified using representative field tests. On the other hand, field evaluations, albeit representative of conditions under which repellents are normally used, can expose volunteers participating in these experiments to mosquito-borne pathogens [6]. Therefore, there is a need to develop methods to test efficacy of repellents under representative user conditions while minimizing volunteer exposure to vector-borne diseases.

There are several techniques that have been proposed for testing topical repellents while reducing human exposure to mosquito bites. These options include: 1) use of synthetic

mosquito attractants that mimic human volunteers [7]; 2) use of animals instead of human volunteers [8,9]; 3) use of *in vitro* blood feeding membrane [10-12]; 4) *In vitro* olfactometry [13]; and, 5) use of a semi-field system (SFS) [14,15]. Although techniques 1 to 4 are convenient because of their high throughput in screening of repellents and do not use human participants, they have well-documented limitations: as the skin is the site of action of topical repellents, and mosquitoes are attracted to cues produced by the host, different hosts will elicit varying degree of responses in the mosquito which will affect both duration and degree of repellency observed [8,10]. The use of *in vitro* blood-feeding membrane is unlikely to give similar results to repellents applied to human skin, as the feeding membrane used in these tests are structurally and physiologically different from the human skin and produce no odour [10]. Use of *in vitro* olfactometry, used mainly to test spatial repellents, is more suitable for screening purposes as it's used in confined spaces and shorter distances in the laboratory and results cannot be correlated to the field, where there are wide open spaces for the mosquitoes to forage [13]. The use of synthetic blends to test repellency has also proved unreliable as different repellent-blend combinations produced disparate results [7]. Use of SFS may overcome these shortcomings because efficacy tests can be performed in a large enclosure under ambient conditions, allowing mosquitoes to elicit similar behavioural responses as under field conditions. The other advantage of SFS is that it uses mosquitoes reared under laboratory conditions and therefore does not expose volunteers to potential mosquito-borne disease. The species, numbers and physiological status of mosquitoes used in the SFS are standardized to provide more controlled conditions and therefore reduce data variability associated with field studies. However, the effectiveness of SFS has not been evaluated against full-field conditions when testing topical repellents. This study examined whether tests carried out in a SFS would yield comparable results to tests conducted in field setting.

2.3 Methods

2.3.1 Study area

Semi-field evaluation of repellents was carried out at Ifakara Health Institute (IHI), Morogoro, Tanzania. The field evaluation of repellents was conducted in Mbingu village, Ulanga district, situated 55 km west of Ifakara town at 8.195°S and 36.259°E. Rapid diagnostic test (RDT) results from passive case detection at a local clinic between December 2012 and July 2013 confirmed malaria incidence estimates from the village were 0.67 cases/person-years, (Jabari Mohammed Namamba, pers comm), only one-and-half years after the end of a national campaign to achieve universal coverage with long-lasting, insecticide-treated bed nets (LLINs) [16]. There is high malaria transmission all year round, with peak transmission occurring in the months of May and June after the long rains. The village experiences an annual rainfall of approximately 1,200-1,800 mm and an annual temperature range of between 20 and 32.6°C. The village borders an extensive field cleared for irrigation, which provides an ideal breeding site for malaria vectors [17].

2.3.2 Semi-field evaluations of topically applied repellents

The semi-field evaluation was carried out in the IHI SFS. A SFS is an enclosed environment, situated in the natural ecosystem of a target vector and exposed to ambient conditions necessary for the completion of the life cycle of the vector. It is made up of a greenhouse frame with walls of mosquito netting and a polyethylene roof, mounted on a raised concrete platform [14,15].

2.3.3 Mosquitoes

The mosquitoes used in these experiments were laboratory-reared *Anopheles arabiensis* (Ifakara strain, originally sourced from Sagamaganga village, Kilombero district in 2008) from the IHI insectaries. The larvae were fed on Tetramin® fish food and maintained at

temperatures of $28 \pm 1^\circ\text{C}$. Pupae were placed in emergence bowls inside a $30 \times 30 \times 30$ cubic cm netted cage in a separate room where temperatures were maintained at $27 \pm 3^\circ\text{C}$ and relative humidity at 70-90%. A 10% glucose solution was supplied in the cages for the emergent adults. The insectary was maintained at 12:12 (light: dark) photoperiod, from 0600 hrs to 1800 hours (light period) and 1800 hrs to 0600 hrs (dark period). The mosquitoes used in these experiments were three to eight day-old nulliparous females. The mosquitoes were starved from sugar solution for six hours.

2.3.4 Volunteers

Male volunteers, aged between 18 and 40 years were educated on aims, benefits and risks of the study and recruited on written informed consent. The use of strictly male volunteers was to prevent potential risk of malaria infection to pregnant female volunteers. All volunteers were highly experienced in performing human-landing catches. During the SFS experiments, volunteers were screened daily for parasitaemia using RDTs and if found positive, excluded from participating any further in the experiments and treated with artemether-lumefantrine (ALU), first-line drug for treatment of malaria in Tanzania. During the field evaluation, in addition to daily screening, volunteers were provided with mefloquine prophylaxis. The volunteers were instructed not to use any fragranced soap or perfume, tobacco or alcohol 12 hours before the start and throughout the experiments.

2.3.5 Repellents

The repellent tested was donated by SC Johnson & Sons Inc (Racine, WI, USA). Three treatments were tested: 1) a lotion-based formulation containing 15% DEET as the active ingredient, being the test product; 2) 15% DEET diluted in absolute ethanol, being the standard control, and 3) a placebo made of a similar lotion formulation as the test product, but

lacking the active ingredient, being the negative control. Technicians were blinded to the repellent application.

2.3.6 Repellent application

To establish the amount of repellent required for application in the SFS experiments, surface area of the lower limbs of three adult male volunteers was determined by first measuring the length from ankle to the knee and the circumference of the ankle and knee using a tape measure. The surface area was then calculated using the formula that expresses the lower limb surface as a trapezium or cylinder:

$$\text{Area} = 0.5(c_a + c_k)D_{ka} \quad (1)$$

where c_a is the circumference of the ankle in cm, c_k circumference of the knee, and D is the distance between c_a and c_k .

Three volunteers were initially asked to apply the repellent *ad libitum* (the amount they felt was safe to protect from mosquito bites) to their legs. While applying the repellent, the volunteers wore latex gloves to avoid absorption of repellents into their skin, which would otherwise reduce the net quantity of repellent applied. The product bottles were then weighed using a precision weighing balance (Ohaus Corp, Pine Brook, NJ, USA) after this initial application to determine the amount applied by each volunteer. The average amount of repellent per volunteer was then calculated from these results. The average amount applied per volunteer was determined to be 2 mg per volunteer-leg. The average surface area of a volunteer's leg was 1,041 cm². The amount of DEET applied was 0.002 mg/cm² (2 mg/1,041 cm²). After amount of repellent required for application was determined, the PI (SO) premeasured these amounts in a Petri dish for each volunteer every evening. The volunteers

were then asked to wear latex gloves and apply their respective amounts on their lower limbs every evening before the start of each experiment.

2.4 Study design

The SFS experiments used a partially randomized, 3×3 unbalanced Latin square design. The three treatments used in these experiments were assigned numbers: 1 (15% DEET lotion), 2 (15% DEET ethanol) and, 3 (placebo lotion). Three volunteers were used in these experiments and were randomly assigned to each of the three treatments using the lottery method. The volunteers were also randomly assigned sitting positions inside the SFS using the lottery method, and moved between the positions in the same order every night. One round of repellent evaluation was made up of three nights of mosquito collections, with each volunteer wearing a different treatment and sitting at a different position on each of these nights. A single set of three volunteers conducted these experiments for six nights (two rounds of repellent evaluation). For logistical reasons, the second set of three volunteers conducted the experiments for three nights (one round of repellent evaluation). Therefore, the mosquitoes were collected for a total of nine nights in the SFS, but with two different sets of volunteers. Data from the three rounds was pooled. The authors are aware that this limitation may have increased data variance because of individual variability in attraction of mosquitoes and efficiency in mosquito collections.

The PI (SO) premeasured the amounts of treatments 15 min prior (17.45), to the start of the experiments and asked the volunteers to apply their respective amounts on their lower limbs while wearing latex gloves. The volunteers had also been asked to put on knee-length shorts and ankle high boots, so as to standardize the area of exposure. The volunteers sat on low stools 10 m equidistant from each other in a triangular formation. A cage holding 100 mosquitoes was placed at the centre of this triangle formation. It was determined from

literature that the biting rate in the study area was 62.5 bites /person/night [18]. Therefore, 100 mosquitoes were released in each hour in the SFS containing three volunteers to simulate the high biting pressure of the field setting. It was assumed that only half the number of all mosquitoes released would bite the volunteers. Therefore, each volunteer would have received approximately 67bites/person/night. The average landing rates/volunteer/hour was also determined. At the top of every hour (18.00 h-22.00) the mosquitoes were released by one of the volunteers. The experiments were conducted from 18.00 because this was the reported time of the start of biting activity of vectors in the study area [19]. In total, four cages containing 100 mosquitoes each were used during each night of the SFS experiment. Each volunteer was given a head torch, which they switched on only when they felt a mosquito landing on their limb or when scanning the legs every 30 seconds for mosquitoes [20]. The volunteers were also given four paper cups, marked from the first to the fourth hour, and instructed to place the catches for each hour in their respective cups. The paper cups were covered with netting that had a hole at the centre to place the mosquitoes into the paper cups, which were plugged using a cotton wool to prevent mosquitoes from escaping. At the end of the experiment (22.00), the mosquitoes collected in the four paper cups were stored in the freezer at the IHI laboratory until the next morning. At 09.00 the next day, the mosquitoes in each paper cup were counted and recorded for each hour. The mosquitoes were then discarded and the paper cups cleaned ready for the day's experiment.

2.4.1 Field evaluation of topically applied repellents

Field evaluation of repellents was conducted in Mbingu village, described above. The experiments were conducted next to the rice fields and away from human dwellings to avoid potential bias in the number and behaviour of mosquitoes [21].

The field evaluation of repellents was conducted using a partially randomized, 3×3 balanced Latin square design, in the same manner as the SFS repellent evaluation described above. All field experiments were conducted at the site identified and described above. Six volunteers, two of whom had also performed the SFS evaluations, were recruited for field evaluation of repellents. A first set of three volunteers conducted the repellent evaluation for nine nights, followed by the second set of volunteers who also conducted the experiment for nine nights at the same site. Therefore six volunteers evaluated the repellents for a total of 18 nights in the field as it was hypothesized that there would be greater variability in field data and more replicates would be required. The volunteers sat 20 m equidistant from each other in a triangular formation. They collected mosquitoes from 18.00 to 22.00, and placed them in the different paper cups marked one to four hours. At the end of the collections, the paper cups holding the mosquitoes were placed in a cool box containing a piece of cotton wool impregnated with chloroform, which killed the mosquitoes. The next morning the mosquitoes in each paper cup were counted by the respective volunteer and the numbers recorded. The mosquitoes were sorted into anophelines and culicines and stored in separate Petri dishes that were layered with cotton wool and silica gel to prevent desiccation. The mosquitoes were brought back to the IHI laboratory where the culicines were identified to species level by an experienced entomologist using taxonomic keys [22]. The *Anopheles gambiae* complex was identified to species level using polymerase chain reaction (PCR) [23].

2.5 Statistical analysis

2.5.1 Calculation of percentage protection

Data from the SFS and field trials were recorded in a Microsoft Excel spreadsheet (Microsoft Corporation), with columns for the date, name of volunteer, treatment the volunteer was wearing, position the volunteer was sitting and the number of mosquitoes caught during each

hour. This data was then exported into STATA 11 (StataCorp LP, College Station, Texas, USA), where the total number of mosquitoes caught when using 15% DEET lotion and 15% DEET in ethanol were compared to the total number of mosquitoes caught when using the placebo lotion for each night regardless of who was using it, and an average was calculated. The reductions in number of mosquitoes in these two treatments (15% DEET lotion and 15% DEET in ethanol) were designated protection and expressed as a percentage, (percentage protection). The formula used to calculate percentage protection is shown below:

$$P = [C-T]/Cx100 \quad (2)$$

where C is the number of mosquitoes caught when the volunteer was using the placebo lotion and T is number of mosquitoes caught when the volunteer was using either the 15% DEET lotion or 15% DEET ethanol.

These results for each night of collection were then aggregated and the average percentage protection when using either 15% DEET ethanol or 15% DEET ethanol calculated using STATA 11.

2.5.2 Poisson regression analysis

Count data was then fitted into a Poisson model in STATA 11, with a log link function and a random intercept for each row of data to account for over dispersion, so as to determine relative risk of being bitten by a mosquito. A Poisson model was chosen because it is used to model count data over a specified period of time, i.e. the number of mosquito bites occurring in one hour. It is also used to model rare events (mosquito bites), which is what was expected when a volunteer was wearing either 15% DEET lotion or 15% DEET ethanol. A Poisson model also allowed for analysis of repeated measures over time on the same individual, i.e. the number of mosquitoes caught by each individual on each day while wearing a different

repellent and sitting at a different position. The number of mosquitoes caught/hour was fitted as the dependent variable, and interaction of repellent with time, individual variability and position fitted as predictors. Day (which also accounted for confounders like temperature, humidity and wind speed), was fitted as a random covariate, and a random intercept, in this case a Unique ID, was fitted into the model to account for over dispersion of the data.

The percentage protection of 15% DEET lotion and 15% DEET ethanol per hour and regression coefficients relative to the placebo (Incidence Rate Ratio, IRR) were determined to assess the decay of repellents through time.

2.6 Ethical considerations

The volunteers used in these experiments were recruited on written informed consent. In case of any positive blood slide for malaria parasites, ALU combination therapy, the first-line drug for malaria treatment in Tanzania, was available. The volunteers were also informed of the study objectives and that they were free to withdraw their participation at any time during the experiments. The volunteers were experienced in human landing catch techniques and were issued with loose net jackets to prevent the mosquitoes biting the upper parts of the body. For field experiments, the volunteers were provided with mefloquine prophylaxis to protect them against contracting malaria. Ethical approval was granted by the Ethical Review Boards of Ifakara Health Institute (IHRDC IRB A46), the Tanzanian National Institute of Medical Research (NIMR/HQ/R8a/VOL IX/780), and London School of Hygiene of Tropical Medicine (LSHTM 5174).

2.7 Results

2.7.1 Semi-field experiments

2.7.1.1 Average percentage protection

The average percentage protection of 15% DEET lotion in the SFS as calculated from Equation 2 above was 82.13% (95% CI 75.93-88.82) and 71.29% (95% CI 61.77-82.28) for 15% DEET in ethanol over four hours of mosquito collection.

2.7.1.2 Poisson regression analysis

The relative risk of being bitten by a mosquito over the four-hour test when using 15% DEET lotion compared to placebo lotion was reduced by 91.8% (95% CI 85.73-95.79%, IRR = 0.082 $z = -8.23$, $P < 0.0001$). When 15% DEET ethanol was compared to the placebo lotion, the relative risk of being bitten by mosquitoes was also reduced by 92.30% (95% CI 85.06-95.45%, IRR = 0.077, $z = -8.21$, $P < 0.0001$) (Table 1). The relative risk of being bitten increased in hours two and three relative to hour one, although these differences were not significant. There was, however, a significant increase in the risk of being bitten in hour four compared to hour one for both 15% DEET lotion IRR = 3.71 (95% CI 1.78-7.78, $z = 3.47$, $P = 0.001$) and 15% DEET ethanol IRR = 3.43 (95% CI 1.60-7.39, $z = 3.17$, $P = 0.002$). This is an indication of repellent decay over time. There was location bias, with position 3 having a higher risk of being bitten compared to location one, IRR 2.00 (95% CI 1.51-2.66, $z = 4.79$, $P < 0.0001$). Position 3 within the SFS was located closest to a nearby restaurant and the mosquitoes were probably more attracted to the light and human cues. There was variability in individual attractiveness to mosquitoes, (Table 2:1).

Table 2:1 Effect of 15% DEET repellent over time, treatment, position and person on *Anopheles arabiensis* in a four-hour repellent evaluation in the semi-field system at Ifakara Health Institute

Treatments	Hours	Incidence rate ratio (IRR) ¹ [95% CI]	Z-test statistic ²	P-value ³
15% DEET in ethanol	1	-	-	-
	2	1.744 [0.796-3.819]	1.39	0.164
	3	1.223 [0.559-2.675]	0.51	0.613
	4	3.708 [1.767-7.780]	3.47	0.001
15% formulated DEET repellent	1	-	-	-
	2	0.877 [0.359-2.140]	-0.29	0.774
	3	1.674 [0.756-3.709]	1.27	0.204
	4	3.439 [1.601-7.386]	3.17	0.002
Treatments				
Placebo	-	-	-	-
15% DEET in ethanol	-	0.082 [0.045-0.149]	-8.23	<0.0001
15% DEET in lotion format	-	0.077 [0.042-0.142]	-8.21	<0.0001
Position				
1	-	-	-	-
2	-	0.818 [0.587-1.139]	-1.19	0.236
3	-	2.000 [1.506-2.656]	4.79	<0.0001
Person				
1	-	-	-	-
2	-	0.619 [0.441-0.868]	-2.78	0.005
3	-	2.372 [1.796-3.133]	6.08	<0.0001

¹ The data for position one, person one and effect of treatments in hour one were used as a reference values for calculating the incidence rate ratios (IRR) for mosquito bites. ² The test statistic z is the ratio of the Coefficient to the Standard error of that respective predictor and is used to test against a two-sided alternative hypothesis that the Coefficient is not equal to zero. ³ The probability (P) that a particular z test statistic is different to what has been observed under the null hypothesis.

Field trial experiments

2.7.1.3 Mosquito species composition in the study area

A total of 4,844 mosquitoes were caught in 72 hours over 18 nights. The catch included: 295 (5.4%) *An. gambiae s.l.*, 3,082 (64.6%) *Mansonia africanus*, 467 (9.8%) *Mansonia uniformis*, 673 (14.1%) *Coquillettidia aureus*, 210 (4.4%) *Culex univattus* and 177 (3.7%) other *Culex* species (Figure 2:1).

Distribution of mosquito species sampled over 18 nights in Mbingu village

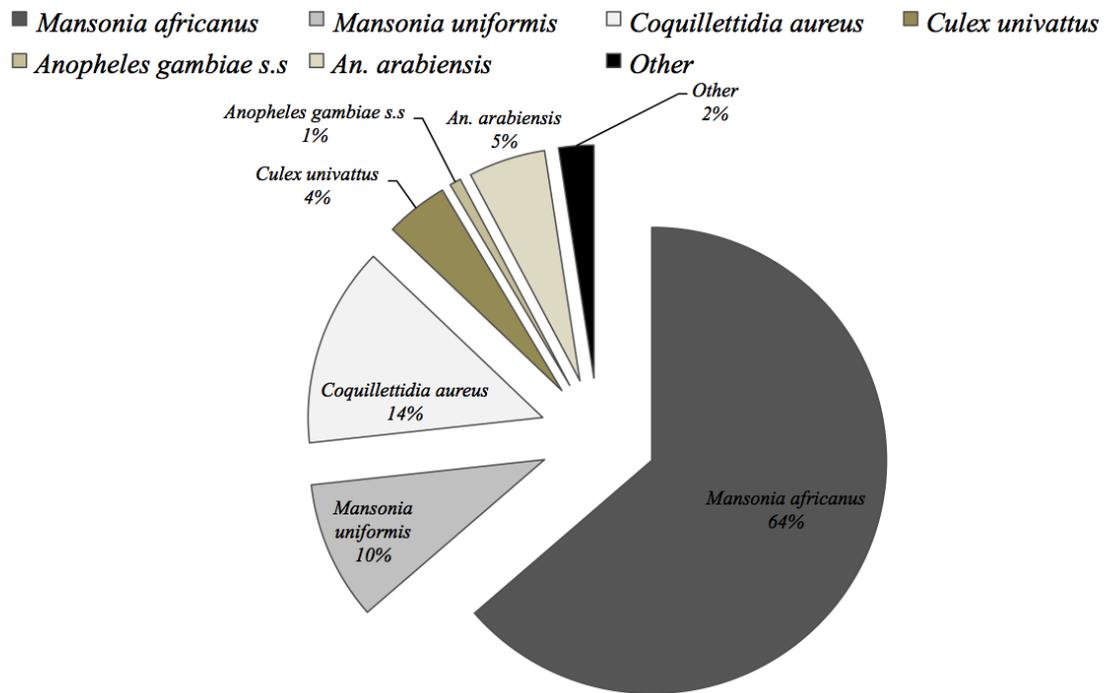


Figure 2:1 Pie chart showing mosquito species composition caught in Mbingu village during human landing catches sampled over 18 nights in field experiments

2.7.1.4 *Anopheles gambiae s.l.* composition in the study area

All the *An. gambiae s.l.* caught were identified to species level by PCR. Out of the 295 successful PCR amplifications, 12.88% (n = 38) were *An. gambiae s.s*, while 87.12% (n = 257) were *An. arabiensis*.

2.7.1.5 Average percentage protection

The average percentage protection, of 15% DEET lotion in the field was 85.10% (95% C.I. 78.97-91.70) and 88.24% (95% C.I. 84.45-92.20) for DEET ethanol over four hours of mosquito collection, as calculated from Equation 2.

2.7.1.6 Poisson regression analysis

The relative risk of being bitten by a mosquito over the four hour test when using 15% DEET lotion was reduced by 94.78% (95% CI 91.46-96.81%, IRR = 0.052, $z = -11.74$, $P < 0.0001$) compared to the placebo lotion and 96.41% (95% CI 93.94-97.88%, IRR = 0.035, $z = -12.42$, $P < 0.0001$) while using 15% DEET in ethanol (Table 2:2).

Table 2:2 Effects of 15% DEET repellent over time, treatment, position and person on total number of mosquitoes in a four-hour repellent evaluation in Mbingu village

Treatments	Hours	Incidence Rate Ratio¹ [95% CI]	Z-test statistic²	P-value³
15% DEET in lotion format	1	-	-	-
	2	0.839 [0.422-1.667]	-0.50	0.618
	3	1.133 [0.578-2.222]	0.37	0.714
	4	1.699 [0.873-3.307]	1.56	0.118
15% DEET in ethanol	1	-	-	-
	2	0.791 [0.381-1.641]	-0.63	0.529
	3	2.049 [1.027-4.090]	2.04	0.042
	4	3.027 [1.524-6.011]	3.17	0.002
Treatments				
Placebo	-	-	-	-
15% DEET in lotion format	-	0.052 [0.038-0.085]	-11.74	<0.0001
15% DEET in ethanol	-	0.035 [0.021-0.060]	-12.42	<0.0001
Position				
1	-	-	-	-
2	-	1.091 [0.851-1.400]	0.69	0.498
3	-	0.876 [0.684-1.123]	-1.04	0.299
Person				
1	-	-	-	-
2	-	4.892 [3.511-6.816]	9.38	0.000
3	-	1.392 [0.973-1.987]	1.81	0.070
4	-	1.065 [0.624-1.820]	0.23	0.815
5	-	0.933 [0.54 0-1.611]	-0.25	0.804
6	-	1.377 [0.808-2.347]	1.18	0.239

¹ The data for position one, person one and effect of treatments in hour one were used as a reference values for calculating the incidence rate ratios (IRR) for mosquito bites. ² The test statistic z is the ratio of the Coefficient to the Standard error of that respective predictor and is used to test against a two-sided alternative hypothesis that the Coefficient is not equal to zero. ³ The probability (P) that a particular z test statistic is different to what has been observed under the null hypothesis.

The risk of being bitten in the fourth hour increased three-fold compared to the first hour when using 15% DEET in ethanol IRR = 3.03 (95% CI 1.52-6.01, z = 3.17, P = 0.001). There was, however, no significant increase in the risk of bitten through hours 1 to 4 when using 15% DEET lotion repellent (Table 2:2). There was lower variability in individual attractiveness to mosquitoes, with only volunteer 2 being significantly more attractive to mosquitoes, IRR = 4.89 (95% CI 3.51-6.82, z = 9.38, P <0.0001). This individual was consistently more attractive in all field experiments. In this field study, the volunteers recruited had differing body mass. There were volunteers who had a larger body mass than

this individual but caught fewer mosquitoes when they were compared. Also, even though all team members were highly experienced, there were more experienced field technicians who did not catch as many mosquitoes as this individual. All volunteers used the same concentration and gram/cm² repellents per body surface area, ruling out the potential bias of one volunteer applying more repellent. Studies have shown variable responses of mosquitoes to singular or constituent host attractive cues. It is therefore likely that, the combination of this volunteers body cues/odours [24], made him more attractive to mosquitoes than the combination of cues that were emitted by the other volunteers.

2.7.1.7 *Anopheles gambiae* experiments

Data on *An. gambiae s.l.* from the study area was analyzed separately to determine the efficacy of repellents on this species of major medical importance.

2.7.1.8 Average percentage protection

The average percentage protection of 15% DEET lotion in the field was 93.40% (95% C.I. 89.21-97.79) and 91.45% (95% C.I. 85.79-97.47) for 15% DEET in ethanol over four hours of mosquito collection, as calculated from Equation 2.

2.7.1.9 Poisson regression analysis

The relative risk of being bitten when using 15% DEET lotion was reduced by 82.86% (95% CI 53.26-93.71, IRR = 0.171, $z = -3.45$, $P = 0.001$) when compared to placebo lotion and by 83.43% (95% CI 55.81-93.79, IRR = 0.165, $z = -3.59$, $P < 0.0001$) when using 15% DEET in ethanol over the four hours of the test. There was no significant difference in the average number of *An. gambiae s.l.* caught at the different positions in the field, in each hour or by each treatment in each hour over the four hours of mosquito collections demonstrating

consistent protection. There was however a significant difference in the average number of *An. gambiae s.l.* caught by volunteer 2: IRR = 2.66 (95% CI 1.42-4.98, $z = 3.06$, $P = 0.002$) and volunteer 6: IRR 0.26 (95% CI 0.81-0.84, $z = -2.25$, $P = 0.025$) relative to volunteer 1 (Table 2:3).

Table 2:3 Effects of 15% DEET repellent over time, treatment, position and person on *Anopheles arabiensis* in a four-hour repellent evaluation in Mbingu village

Treatments	Hours	Incidence rate ratio (IRR) ¹ [95% CI]	Z-test statistic ²	P-value ³
15% DEET in lotion format	1	-	-	-
	2	0.403 [0.083-1.956]	-1.13	0.260
	3	0.326 [0.068-1.550]	-1.41	0.159
	4	0.722 [0.185-2.812]	-0.47	0.639
15% DEET in ethanol	1	-	-	-
	2	1.229 [0.343-4.399]	0.32	0.750
	3	1.963 [0.583-6.621]	1.09	0.277
	4	1.370 [0.400-4.693]	0.86	0.500
Treatments				
Placebo	-	-	-	-
15% DEET in lotion format	-	0.171 [0.063-0.467]	-3.45	0.001
15% DEET in ethanol	-	0.165 [0.062-0.441]	-3.59	<0.0001
Position				
1	-	-	-	-
2	-	0.932 [0.542-1.602]	-0.25	0.800
3	-	1.262 [0.750-2.126]	0.88	0.380
Person				
1	-	-	-	-
2	-	2.660 [1.420-4.979]	3.06	0.002
3	-	1.801 [0.924-3.510]	1.73	0.084
4	-	0.381 [0.127-1.141]	-1.72	0.085
5	-	0.328 [0.106-1.015]	-1.93	0.053
6	-	0.262 [0.081-0.841]	-2.25	0.025

¹ The data for position one, person one and effect of treatments in hour one were used as a reference values for calculating the incidence rate ratios (IRR) for mosquito bites. ² The test statistic z is the ratio of the Coefficient to the Standard error of that respective predictor and is used to test against a two-sided alternative hypothesis that the Coefficient is not equal to zero.

³ The probability (P) that a particular z test statistic is different to what has been observed under the null hypothesis.

2.7.1.10 Comparison of full field and semi-field system data

Decay of repellent from the Poisson regression equations (Tables 2:1 and 2:2) and the linear regression demonstrated that 15% DEET in lotion format decayed at a slower rate than 15% DEET in ethanol in both the SFS and field settings. A linear regression also demonstrated a similar trend with regression coefficients showing a more rapid decay of 15% DEET in ethanol in the SFS and against all mosquitoes in the field, with equal decay of the two formulations against *An. gambiae s.l.* in the field (Table 2:4). However, the results from the linear regression equations (regression coefficients) should be interpreted with caution as the data were over dispersed even after transformation to a proportion (percentage protection) and also linear regression is a parametric test that assumes equal variance around the mean. The percentage protection provided by 15% DEET lotion and 15% DEET in ethanol was similar in the SFS and field settings and on both occasions both treatments provided greater protection in the field than in the SFS (Figure 2). When the two treatments (15% DEET lotion and 15% DEET ethanol) were compared statistically there was no difference between the two measured in the SFS IRR = 0.904 (95% C.I. 0.44-2.80, p = 0.833) or the field IRR = 0.621 (95% C.I. 0.316-1.221, p = 0.168).

Table 2:4 Comparison of rate of decay of repellents, percentage protection and log-transformed means of mosquito catches per hour in the semi-field system against *Anopheles arabiensis* and in the field against all mosquito species and *Anopheles arabiensis*

Experiment	Hour	Regression equation	Treatments	GEOMEAN	Percentage protection (CI)*
Semi-field evaluation against <i>An. arabiensis</i>	1	$Y = -0.0765x + 1.0315$ $R^2 = 0.29138$	Lotion-based 15% DEET repellent	2.69	90.88 (84.25-98.03)
	2			1.7	91.85 (84.85-99.43)
	3			3.1	82.60 (70.39-96.93)
	4			4.63	65.97 (52.28-83.24)
	1	$Y = -0.119x + 0.9685$ $R^2 = 0.08181$	15% DEET in ethanol	4.65	75.55 (51.79-110.20)
	2			3.63	70.76 (54.63-91.65)
	3			3.17	82.18 (61.19-110.36)
	4			6.26	58.42 (40.45-84.36)
Field evaluation against all mosquito species	1	$Y = -0.0077x + 0.8921$ $R^2 = 0.00174$	Lotion-based 15% DEET repellent	4.77	87.39 (76.49-99.83)
	2			4.03	88.92 (79.15-99.88)
	3			5.44	85.99 (76.30-96.90)
	4			8.03	83.98 (73.78-94.19)
	1	$Y = -0.0427x + 1.0009$ $R^2 = 0.11871$	15% DEET in ethanol	4.22	91.98 (84.14-100.55)
	2			5.94	95.11 (91.02-99.37)
	3			10.89	87.87 (83.08-92.95)
	4			13.5	79.03 (69.14-90.33)
Person Field evaluation against <i>An. arabiensis</i>	1	$Y = 0.0311x + 0.7904$	Lotion-based 15% DEET repellent	1.22	92.58 (83.18-103.05)
	2			1.25	100.00 (100.00-100.00)
	3			1	92.60 (84.30-101.72)

		0.06763		
4			1.64	88.02 (76.15-101.75)
1	$Y = 0.0208 + 0.6235$	15% DEET in ethanol	0.72	95.20 (87.33-103.78)
2			0.94	94.93 (87.85-102.57)
3	$R^2 = 0.045263$		1.5	82.26 (61.18-110.61)
4			1.17	91.15 (83.82-101.31)

- Some confidence intervals exceed 100% because the ranges were calculated by regression analysis using continuous data. They should therefore be read as 100% efficacy

Comparison of percentage protection of 15% Deet lotion and 15% Deet in ethanol against *An.arabiensis* in the SFS, all mosquito species in the field and *An. arabiensis* in the field during 4 hours of mosquito collection

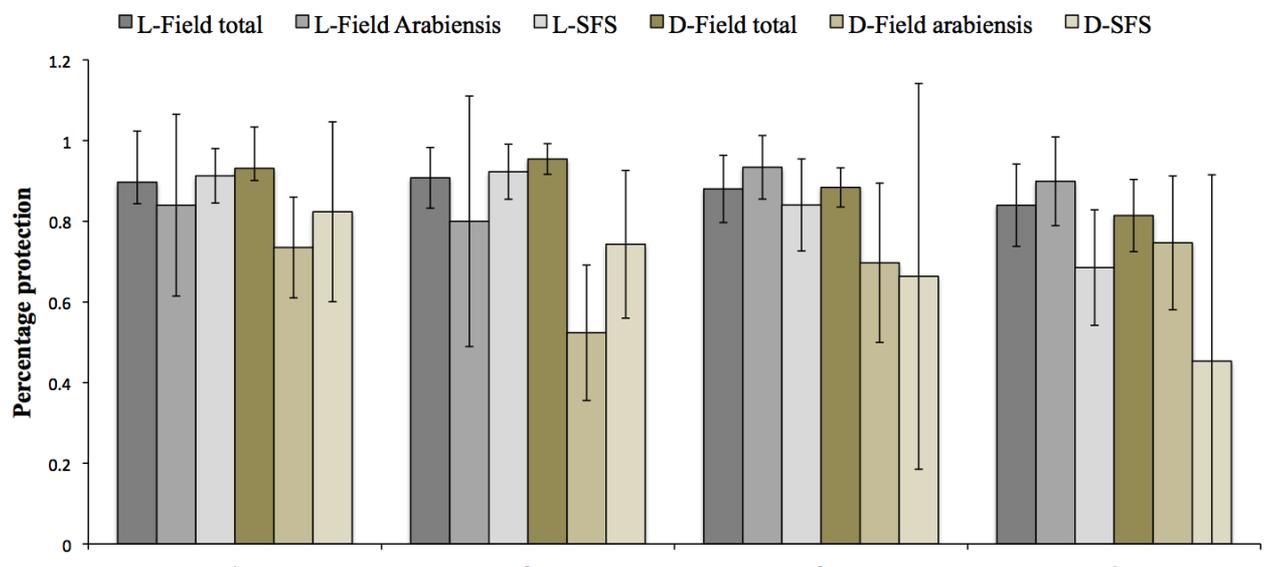


Figure 2:2 Comparison of percentage protection of 15% DEET lotion and 15% DEET ethanol against *An. arabiensis* in the semi-field system, all mosquito species and *An. arabiensis* in the field after four hours of mosquito collection

*L-Field total is 15% DEET lotion tested against all mosquito species in the field. L-Field *Arabiensis* is 15% DEET lotion against *An. arabiensis* in the field. L-SFS is 15% DEET lotion against *An. arabiensis* in the semi-field system. D-Field total is 15% DEET in ethanol tested against all mosquito species in the field. D-Field *Arabiensis* is 15% DEET in ethanol

against *An. arabiensis* in the field. D-SFS is 15% DEET in ethanol against *An. arabiensis* semi-field system.

2.8 Discussion

The epidemiology of malaria in sub-Saharan Africa is experiencing a subtle shift. Before the advent of LLINs and indoor residual spraying (IRS), malaria transmission was mediated indoors and late in the night mainly by *An. gambiae s.s.* This species of the *An. gambiae* complex is known to be predominantly anthropophilic, endophagic and endophilic [25,26]. This characteristic is responsible for the success of LLINs and IRS in controlling *An. gambiae s.s.*, as these tools predominantly target indoor biting and resting malaria vectors. However, *An. arabiensis*, the other dominant vector species of the *An. gambiae* complex [26] exhibits a more plastic behaviour [27]. In areas where the host is predominantly human and found indoors, this vector displays anthropophilic, endophagic and endophilic behaviour, similar to its sibling species, *An. gambiae s.s.* However in areas where the host is found outdoors and is non-human, *An. arabiensis* readily shifts to exophagic, exophilic and zoophagic behaviour [25]. Therefore, extensive and long-term employment of LLINs and IRS is likely to significantly diminish and in some situations completely eliminate the populations of *An. gambiae s.s.*, thereby selecting for the highly adaptable *An. arabiensis* that predominantly bites early in the evening and outdoors [27]. As a result, even though LLINs and IRS will decrease malaria transmission as a whole, there will be a substantial proportion of residual transmission occurring outdoors and in the early evenings that these intradomestic tools cannot tackle [27].

Consequently, there is a need to develop novel tools or methods that can tackle this residual transmission. Repellents, both topical and spatial, provide a promising solution for controlling outdoor transmission [28-30]. However before topical

repellents are employed in the community, their performance needs to be correctly and accurately measured under user conditions. It is, therefore, essential to develop a robust methodology for testing repellent efficacy that is representative of conditions under which the repellents are used (the community), but does not expose individuals conducting these experiments to potential malaria vectors [1,2]. It was hypothesized that locating the SFS in regions representative of ambient conditions for the targeted disease vector and testing repellents on humans against these vectors is likely to yield results that correlate well with field tests. Therefore, to qualify the effect of these treatments in these two settings, data for *An. arabiensis* in the SFS was analyzed against data of *An. gambiae s.l.* in the field experiments (as > 80% of this species complex was found to be *An. arabiensis*).

The findings demonstrated that 15% DEET lotion protected against 82.13% (95% CI 75.93-88.82) of the bites in the SFS compared to 93.40% (95% C.I. 89.21-97.79) protection against bites in the field, while 15% DEET in ethanol protected against 71.29% (95% CI 61.77-82.28) bites in the SFS compared 91.45% (95% C.I. 85.79-97.47) bites in the field against *An. gambiae s.l.* These results demonstrate that both 15% DEET lotion and 15% DEET repellent were more efficacious in the field than in the SFS. A plausible explanation for this might be the high biting pressure observed in the SFS compared to the field. Mosquitoes were exposed to fewer hosts than they normally would in the field and their numbers were continuously increased from 100 mosquitoes in the first hour to 400 mosquitoes in the fourth hour (Table 2:5 & 2:6). By simulating high biting pressure that increased over time as is seen in the field due to the circadian rhythm of the local malaria vectors [19], the authors ensured that the repellent worked extremely well against the predominant malaria vector species before going to the more dangerous field setting. It is known that repellents have

varying effects on the other mosquito species present in the field [6,31]. As a result, the effect of the repellent in the field might be over or underestimated depending on the other species present in the field. It is, therefore, prudent, that before the effect of a repellent is established, it should be tested against different mosquito species to assess its efficacy. These data showed that DEET efficacy against one Anopheline species only in the SFS was similar to that for a range of non-anophelines in the full field although this needs to be validated for other repellent classes, as not all repellents are broad-spectrum.

Table 2:5 Mean landing rates (MLR) of *Anopheles arabiensis*/volunteer/hour in a four-hour repellent evaluation in the Semi-field system at Ifakara Health Institute

	Volunteer 1 Median (IQR)	Volunteer 2 Median (IQR)	Volunteer 3 Median (IQR)
<i>Placebo</i>			
Hour 1	17 (6–20)	22 (11–27)	41 (19–46)
Hour 2	16 (13–19)	18 (8–18)	17 (16–43)
Hour 3	14 (10–24)	24 (6–29)	37 (18–56)
Hour 4	14 (11–30)	16 (8–20)	28 (12–36)
<i>15% DEET in ethanol</i>			
Hour 1	0	0	12 (1–13)
Hour 2	1 (0–3)	1 (0–5)	8 (7–10)
Hour 3	1 (0–1)	0 (0–1)	9 (6–19)
Hour 4	4 (1–10)	4 (0–4)	19 (7–18)
<i>15% DEET in lotion formulation</i>			
Hour 1	2 (0–4)	0 (0–1)	4 (2–6)
Hour 2	1 (1–5)	2 (0–2)	1 (1–5)
Hour 3	3 (2–15)	2 (0–2)	3 (2–4)
Hour 4	3 (2–17)	3 (2–5)	8 (4–10)

Table 2:6 Mean landing rates (MLR) of *Anopheles gambiae s.l.*/volunteer/hour in a four-hour repellent evaluation in Mbingu village

	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4	Volunteer 5	Volunteer 6
	Median	Median	Median	Median	Median	Median
	(IQR)	(IQR)	(IQR)	(IQR)	(IQR)	(IQR)
<i>Placebo</i>						
Hour 1	10 (2–10)	2 (0–3)	4 (1–5)	0 (0–2)	0 (0–6)	(0)
Hour 2	2 (1–7)	4 (2–4)	3 (1–4)	2 (0–5)	1 (0–3)	1 (0–3)
Hour 3	4 (1–22)	3 (0–6)	10 (1–13)	0 (0–3)	0 (0–4)	0 (0–4)
Hour 4	4 (0–6)	3 (1–7)	11 (3–12)	0 (0–8)	2 (0–3)	0 (0–5)
<i>15% DEET in ethanol</i>						
Hour 1	0 (0–0)	2 (1–9)	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)
Hour 2	0 (0–1)	1 (0–7)	2 (0–6)	0 (0–0)	0 (0–0)	0 (0–0)
Hour 3	0 (0–3)	4 (1–8)	2 (0–4)	0 (0–5)	0 (0–0)	0 (0–0)
Hour 4	0 (0–0)	4 (1–5)	3 (1–6)	0 (0–0)	0 (0–0)	0 (0–1)
<i>15% DEET in lotion</i>						
Hour 1	0 (0–0)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–2)	1 (0–1)
Hour 2	0 (0–0)	0 (0–2)	0 (0–0)	1 (0–1)	0 (0–0)	0 (0–0)
Hour 3	0 (0–1)	1 (0–1)	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–0)
Hour 4	0 (0–0)	2 (0–3)	0 (0–2)	0 (0–0)	0 (0–0)	0 (0–0)

It is often assumed that formulated repellents provide longer protection against arthropod bites, especially those that have a high vapour pressure. However, findings from this study demonstrate that this may not always be true, and that different formulations of repellents containing the same amount of active ingredient (AI) provide relatively similar efficacy against arthropod bites. These findings are similar to a study carried out to test the efficacy of different formulations of repellents against ticks [32,33].

This is the first study known to have compared the efficacy of topical repellent in both the SFS and field and to determine a correlation between these two settings. However, the current study did suffer from some shortcomings, and an attempt to outline a

rationale procedure for conducting future studies incorporating the lessons learnt from this study is suggested below.

A fully randomized, balanced Latin square design should be employed, so that each volunteer tests each of the repellents in all positions available in the SFS. Each volunteer should test each treatment for an equal number of days in each position. The treatments and positions should be randomly assigned to the volunteers and the movement through these positions should be also be randomized. The exact number volunteers testing the repellents should be established, and this number used to calculate the average repellent dose to be applied per individual/surface area. This is to avoid under or overestimating the repellent dose required per person in a case where fewer or more individuals are used to establish the amount of repellent required than those actually testing the repellents. Each group of volunteers testing the repellents should perform an equal number of replicates so that the results are not confounded by individual variability in attractiveness of mosquitoes, a bias that is minimized when all volunteers have equal number of replicates. All repellent application should be done by an individual wearing gloves, either by the volunteers themselves or an assistant, to prevent repellent absorption into the skin, thereby reducing net amount of repellent being applied. The local dominant vector species, the biting rate per night and time of biting should be established and the number of mosquitoes representative of the biting rate used in the study. The experiments should also be started at the beginning of peak biting activity of the dominant vector in the local area, to avoid interfering with the circadian rhythm. Varying the biting pressure and peak biting times may vary the results of the SFS.

Using a new model of repellent efficacy as a function of user compliance and malaria intensity developed by SJM and Briet (personal communication), the predicted reduction in malaria provided by the repellent in this scenario would be 44%, assuming 80% repellent efficacy and 80% compliance among users with a sporozoite index of 0.005637 (Okumu, personal communication), a transmission season of 200 days per year and biting pressure of 32 bites per night from the major malaria vector *An. arabiensis* [34].

2.9 Conclusion

The findings of this study support the hypothesis that repellent testing conducted in SFS yields similar results to field tests, and could be used in place of field tests, to avoid unnecessary exposure of volunteers to potentially infectious disease vectors, provided repellent efficacy is established against a range of representative mosquito species.

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Chapter 3: A cluster-randomized controlled trial to assess the effectiveness of using 15% DEET topical repellent with long-lasting insecticidal nets (LLINs) compared to a placebo lotion on malaria transmission



3.1 Abstract

Background

Long-lasting insecticidal nets (LLINs) have limited effect on malaria transmitted outside of sleeping hours. Topical repellents have demonstrated reduction in the incidence of malaria transmitted in the early evening. This study assessed whether 15% DEET topical repellent used in combination with LLINs can prevent greater malaria transmission than placebo and LLINs, in rural Tanzania.

Methods

A cluster-randomized, placebo-controlled trial was conducted between July 2009 and August 2010 in a rural Tanzanian village. Sample size calculation determined that 10 clusters of 47 households with five people/household were needed to observe a 24% treatment effect at the two-tailed 5% significance level, with 90% power, assuming a baseline malaria incidence of one case/person/year. Ten clusters each were randomly assigned to repellent and control groups by lottery. A total of 4,426 individuals older than six months were enrolled. All households in the village were provided with an LLIN per sleeping space. Repellent and placebo lotion was replaced monthly. The main outcome was rapid diagnostic test (RDT)-confirmed malaria measured by passive case detection (PCD). Incidence rate ratios were estimated from a Poisson model, with adjustment for potential confounders, determined *a priori*. According-to-protocol approach was used for all primary analyses.

Results

The placebo group comprised 1972.3 person-years with 68.29 (95% C.I 37.05-99.53) malaria cases/1,000 person-years. The repellent group comprised 1,952.8 person-years with 60.45 (95% C.I 48.30-72.60) cases /1,000 person-years, demonstrating a

non-significant 11.44% reduction in malaria incidence rate in this group, (Wilcoxon rank sum $z = 0.529$, $p = 0.596$). Principal components analysis (PCA) of the socio-economic status (SES) of the two groups demonstrated that the control group had a higher SES (Pearson's chi square = 13.38, $p = 0.004$).

Conclusions

Lack of an intervention effect was likely a result of lack of statistical power, poor capture of malaria events or bias caused by imbalance in the SES of the two groups. Low malaria transmission during the study period could have masked the intervention effect and a larger study size was needed to increase discriminatory power. Alternatively, topical repellents may have no impact on malaria transmission in this scenario. Design and implementation of repellent intervention studies is discussed.

3.2 Background

In the past decade, considerable financial and political resources have been mobilized for malaria control [1]. This has in turn led to extensive coverage and use of existing control tools, like long lasting insecticidal nets (LLINs) and indoor-residual spraying (IRS) [1]. Implementation of these highly effective vector control tools has resulted in substantial decrease in malaria transmission, morbidity and mortality [2-4]. Despite both extensive coverage and use, the sole use of these tools has not and will not be able to eliminate malaria in all malaria endemic regions [5]. Because LLINs and IRS target mainly indoor biting and indoor resting vectors their implementation may select for outdoor resting and biting vector populations that often become dominant, so that even though there is a diminished malaria transmission as a result of extensive LLINs and IRS use, there is likely to be a larger proportion of this residual transmission occurring outdoors compared to indoors [6].

Increased urbanization and rural electrification programmes have also had an impact on malaria transmission dynamics. As a result of this, individuals stay up later in the evenings than they usually would in a situation where electricity was not available [7], and are, therefore, exposed to potentially infective mosquito bites for longer.

With the renewed push for malaria elimination [8], it is evident that new tools need to be developed to augment existing vector control tools to achieve this goal. Topical repellents provide excellent personal protection [9] and could potentially be used to complement LLINs for additional protection from residual transmission [5]. Several studies demonstrated that topical repellents offer additional protection from malaria transmission either when used alone, or in combination with LLINs, in areas with high early evening and outdoor malaria transmission [10-12].

This study assessed the potential additional benefit of using topical repellents in combination with LLINs compared to using only LLINs on early evening malaria transmission in a rural community in Kilombero valley, south-west Tanzania.

This community mainly relies on subsistence farming of rice, which provides for a large breeding site for both malaria vectors and nuisance biting mosquitoes [13]. It is customary that the community in the study area cooks outdoors in the early evenings, a situation that is likely to expose them to mosquito bites and potential malaria transmission. Rural development is also rapidly taking place in this study area. As a result, many members of the community usually gather in the early evening and stay late into the night at local entertainment spots that are springing up in the study area owing to rural electrification programmes, thereby increasing the potential of malaria transmission at these times. A recent report estimates a malaria incidence rate of 0.67 cases/person/year confirmed by rapid diagnostic test (RDT) from passive case detection at a local clinic between December 2012 and July 2013 (Jabari Mohammed Namamba, pers. comm.).

In the past two decades, extensive malaria intervention programmes have taken place in this area, and it is therefore expected that the community be highly sensitized on malaria transmission and control methods [14-17]. There is high LLIN use in the study area [18]. Repellent awareness and knowledge as assessed using a Knowledge, Attitude and Practice (KAP) baseline questionnaire at the inception of the clinical trial determined that this community did not use topical repellents as a mosquito control tool. Awareness and availability were reported as the major reasons for not using topical repellents

The major malaria vector in the study area is *Anopheles arabiensis* [19], which has been shown to exhibit elastic feeding behaviour depending on the availability and location of the host [6] and is known to exhibit early evening biting [20]. The dominance of this vector in this area is also likely to be the result of extensive LLIN use in the study area [21,22].

A field study conducted in the study area to determine the efficacy of this repellent (15% DEET) against *An. arabiensis* demonstrated >80% protection from bites over four hours of mosquito collection [19]. Therefore, 15% DEET was considered appropriate to provide protection against early evening biting.

This study area was chosen because there are no studies that have been conducted to assess the additional benefits of topical repellents to LLINs in malaria control in East Africa, although this technology has been shown to work elsewhere in sub-Saharan Africa [23,24]. Also, the vectors present in the area, *An. arabiensis*, exhibit early evening biting [20], a trait that made the use of repellents in the early evening ideal in this area. Therefore, even though extensive employment of current control tools will lower malaria transmission in this area, it is likely that residual transmission will continue occur at times when the effectiveness of these tools is diminished, like outdoors in the early evenings and mornings, [6] and will require supplementary tools that target this scenario.

Therefore, it was hypothesized that combined use of LLINs and topical repellents in this community would have a greater impact on malaria transmission in the early evening compared to sole use of LLINs.

3.3 Methods

3.3.1 Study area

The study was carried out in Mbingu village, Ulanga district, situated 55kms west of Ifakara town at 8.195°S and 36.259°E. At the time of the study inception, (July 2009), the village was estimated to have 7,609 inhabitants [25]. There is moderate malaria transmission in the study area, with peak transmission occurring in the months of May and June after the long rains. The village experiences an annual rainfall of approximately 1,200-1,800 mm and an annual temperature range of between 20 °C and 32.6 °C. The village borders an extensive field cleared for rice irrigation, which provides an ideal breeding site for malaria vectors [13].

3.3.2 Sample size rationale

The only available data from the study area were community reported fever incidence rate estimates of 3.2 cases/person/year for children under the age of five years [26]. Assuming fever rates in children under five years are higher than the rest of the population, and that not all fevers reported are caused by malaria, a rate of one malaria case/person/year was used to calculate the sample size needed for this study. Available reports also indicated that 30% of mosquito bites occurs in the early evening [20]. Therefore, assuming that mosquitoes have an equal probability of carrying sporozoites regardless of time of night, it was assumed there was a potential 30% malaria transmission occurring in the early evenings. Expecting that repellents would reduce 80% of this potential 30% early evening transmission, as observed from the field study [19], it was reasoned that repellents would reduce the overall transmission of malaria from one case/person/year to 0.76 cases/person/year. Using the methods of Hayes *et al.* [27] for sample size calculation for cluster randomized

trials, it was estimated that to observe this treatment effect (24%), with 90% power at the two-tailed 5% significance level, 10 clusters of 47 households with five members each was required per treatment group. A coefficient of variation (k) of 0.20 was used based on published recommendations, as the inter-cluster variation could not be estimated [28].

3.3.3. Household recruitment

Households were recruited into the study in two phases. In phase one, the study investigators and field team visited the study village for reconnaissance and introduction to the community leaders and members in December 2008. A week later, the study team returned to the study village and aided by community leaders, identified the centre of the village. Here, the field team spun a ballpoint pen and visited all the households that the writing end of the pen pointed to with the intention of recruiting all consenting households into the study. After all households in this direction had been exhausted, the field team went back to the village centre and spun the pen to choose the next direction in which to visit the households. If the pen pointed in the direction where the households were already visited, then, the pen was spun again until a new direction was identified. This progression was repeated until approximately, 1,000 households had been visited and recruited. The village had 2,000 households [25] and, therefore, by visiting and potentially enrolling at least 50% of the households, the study team were confident that they had captured a representative sample of households in the study area.

3.3.4 Enrolment of households into the study

During the household recruitment visits, each household head was informed of the purpose of the visit. They were educated on the objectives, risks and benefits of the

study to their household and the community. They were encouraged to ask questions and after all their concerns had been addressed, they were asked if they were willing to participate in the study. If willing, each household head was asked to sign a written informed consent form, confirming their participation and that of all household members. As data was being collected at the household level, only the household head was asked for informed consent. It was assumed that once that household head gave consent then all household members would likely comply with repellent use following instructions of the household head as the authority in each household. A structured questionnaire on the socio-economic status (SES) of the household and knowledge, attitude and practice (KAP) in relation to malaria and repellents was then administered. The GPS coordinate of the household enrolled was then recorded using a handheld GPS receiver (Garmin eTrex Legend® H). These coordinates were then plotted using Arc GIS software (Arc GIS 9.0, ESRI, UK), to generate a map of all the households enrolled in the study area.

3.3.5 Second phase of household recruitment, household enrolment and cluster generation

In phase two, the map generated during the first phase of recruitment was used to delineate 20 clusters of households, while ensuring a buffer zone of 200 metres between clusters to prevent diversion of mosquitoes from the intervention group to the control group. As a result of creation of this buffer area, some households that had been recruited in the first phase fell within this 200 meter buffer area. These households were excluded from the study during this second phase of recruitment. Therefore, even though about 1,000 households were recruited in the first phase, more households needed to be recruited in the second phase as a result of loss of households within the buffer area. These households were excluded because they

would have potentially confounded the outcome of the study in case of diversion of mosquitoes. All households within the buffer area were issued with an LLIN per sleeping space to protect them from potentially greater than normal bites from diverted mosquitoes. In practice, the second phase of recruitment proceeded as follows: The field team visited the 20 clusters, using the household considered to be at the centre of these clusters (identified from the Arc GIS map), as the starting point. The household head of the central household in the cluster was informed of the purpose of the visit. If the household had been enrolled during the first phase of household recruitment, then the field team issued an LLIN for every sleeping space, stapled a unique identifier number on the doorframe and moved to the next nearest household. If the households had not been enrolled, the household head was informed of the objectives, risks and benefits of the study, enrolled on written informed consent, provided with a unique household identifier and LLINs for each sleeping space, and a SES and KAP questionnaire administered. This progression was repeated until 47 households close together were enrolled to form a single cluster. All 47 households in each of the 20 clusters were enrolled in this manner. The newly enrolled households that did not appear on the map generated in the first phase of recruitment were plotted and the map updated to produce the final map of households recruited into the study (Figure 3:1).

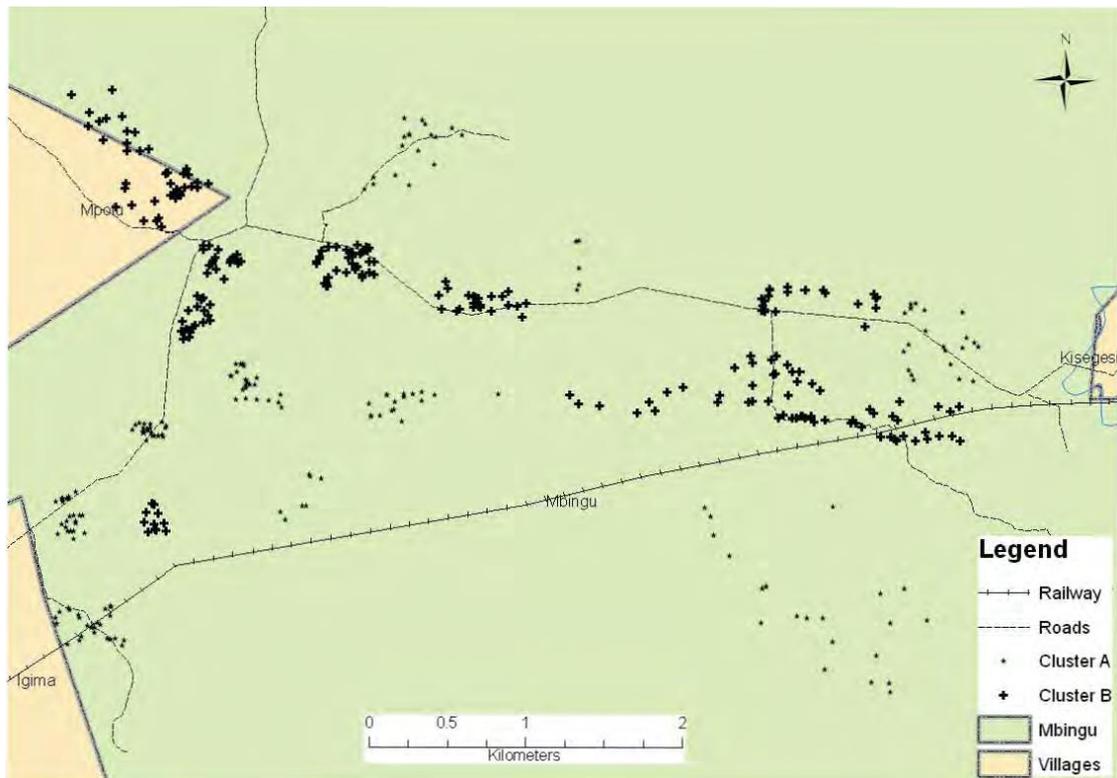


Figure 3:1 Map of household recruited into the trial in the study village

Clusters were used as the unit of randomization for three reasons: 1) since the intervention would be applied to a community, if proven to be effective, 2) to limit contamination of treatments between households, and 3) to avoid diversion of mosquitoes from individuals who used repellents to those who did not use repellent within the same household or from households using repellents to households that used the placebo, thereby putting non-repellent using individuals and households at a potentially higher risk of contracting malaria [29,30].

3.3.6 Eligibility criteria

All households were eligible to be recruited into the trial and no household was excluded on the basis of household structure, asset or livestock ownership. All individuals older than six months of age were eligible to be recruited into the trial. This age cut-off was used because re evaluation of DEET insect repellent [31]

estimated the margin of exposure (MOE) in children less than six months to be less than 100. Margin of exposure is defined as the ratio of dose of DEET used daily to the no observed effect level dose recommended by regulation agencies, which usually consider doses, which result in MOEs of less than 100, unacceptable. Based on this risk assessment, use of DEET was not recommended for children under six months [32].

3.3.7 Randomization of clusters to treatments

All the 20 clusters in the map (Figure 3:1) were assigned numbers 1 to 20, starting from the left hand side to the right. The cluster numbers were then written down on small pieces of paper, which were placed in a bowl. The principal investigator (PI) and project leader (PL) then drew the pieces of paper from the bowl one at a time. Two three digit numbers (258 and 305) were used to classify clusters in to two groups. The first cluster number to be drawn was assigned treatment 258 and the second cluster number assigned treatment 305. This progression was repeated until all the clusters had been assigned to one of the two groups.

3.3.8 Blinding

The repellent and placebo lotion smelt and felt the same and were placed in identical tubes, distinguishable only by the two three-digit numbers known only to the independent code keeper (SC Johnson and Sons). However, the PI and PL had previously conducted efficacy test of these two treatments [19], and could identify the repellent and placebo from the results of this study. Therefore, it was only the field team, study statistician and study participants who were blinded in this study. Blinding was broken after analysis.

3.3.9 Repellent issuance, application and compliance

In June 2009, the field team visited all households enrolled in the study to distribute treatments to study participants. The treatments, (15% DEET and placebo), both formulated as a pourable lotion that is applied by hand, were supplied by SC Johnson, Racine, USA, and packaged in 100 ml plastic tubes. During this visit, the field team informed the household members on how to apply the treatments provided on exposed areas of the body. They also advised the participants not to apply the treatments on open wounds, eyes, mouth and areas with mucous membranes. The repellent lotion was applied at an approximate rate of 0.002 mg DEET / cm², the quantity of repellent that prevented >80% mosquito bites for 4 hours in a controlled environment and in the study area [19]. Even though a repellent with a higher concentration would have provided greater protection, the Tanzania National Institute of Medical Research ethical approval board did not allow the use of a repellent that had more than 15% DEET due to safety concerns, despite the initial request of the PI to use 30% DEET and submission of detailed experimental justification and dossier of safety data justifying the use of a higher concentration.

The participants were issued measuring caps, with amounts of repellent required for adults (7mls) and children below 12 years (3mls) marked on the cap. Each tube held 100mls of repellent. Therefore, two tubes were considered enough to last an adult one-month, i.e. if they applied the recommended dosage of 7 mls per day, while one tube was enough to last a child < 12 years for one month, if they used 3mls per day. Children > 12 years were advised to use up to 7mls a day, and were therefore issued with 2 tubes for the month. All the tubes issued per cluster and households were identical, and it is possible that the household members shared a single tube of repellent until it ran out. As all households member were issued with enough

treatment to last them month, either 15% DEET repellent lotion or placebo, and dosages for adults and children had been marked out, it was assumed that sharing of repellents within the household would have no effect on the outcome as long as there was daily compliance to the recommended dose by the participants. The amounts recommended were adjusted to accommodate for individuals with greater than average body mass as it was determined from semi-field and field experiments that an average sized volunteer required 6 mls [19]. This amount was, therefore, adjusted upwards by an extra milliliter. The community members were instructed to apply the repellent at dusk (1800 hrs) and to reapply it if they felt any mosquito bites or remained active for more than four hours after sunset.

Compliance to lotion use (both repellent and placebo) was assessed by the field team visiting the enrolled households at the beginning of each subsequent month (monthly monitoring surveys) to issue new tubes of repellent and placebo lotion. Therefore compliance was assessed on a monthly basis using a short structured questionnaire, where the household head or an adult household member, was asked if all household members had used the repellents and reasons for non-compliance where relevant. However, as self-reported data are unreliable, the number of repellent/placebo tubes issued every month was also recorded as a secondary measure of compliance, to determine if there was a difference in the number of tubes issued in each month per treatment group. Data on use of LLINs the previous night, malaria infection, recalled febrile illness and visit to the health centre during that month was also collected. If, during these monthly monitoring surveys, the household head or any other adult household member was not available to answer the questionnaire on compliance, the field team visited that particular household daily for seven consecutive days. If still no household member able to take the monitoring survey was available during these

repeated visits, then that household, and all its members, was excluded from the calculation of person-time for that month.

In addition to the compliance, malaria and recalled febrile illness data collected during each month of the study period, an after study questionnaire was administered at the close of the study to assess the participants' knowledge, attitudes and practice in relation to repellents. These results are reported elsewhere.

3.3.10 Clinical data collection

A single government health facility in the study area was recruited into the study. At this facility, health services were provided for free by the project if the participants showed their project identification card with a household unique identification number on it. Community members that were not enrolled into the study were issued with a different kind of identification card to also allow them free consultation and treatment at the recruited health facility. This was done to discourage community members attending the health facility under the guise of being a study participant and, therefore, contaminating the study by recording malaria status of community members not enrolled in the study as participants. It was assumed that since services were provided for free at this facility, it would attract most community members seeking health services. A clinical officer (CO) and a nurse were employed by the project at this health facility. A ledger with the household unique identifier and names of each household member was drawn up and placed at this health facility. When a study participant visited the health facility with febrile illness, the CO checked against their name and household unique ID in the health facility ledger. This way household and health facility data could be reconciled using the household unique identifier. Febrile participants were tested for malaria using rapid diagnostic test (RDT) (ICT

Malaria cassette tests HRPII/pf test kit). A proportion of participants also had diagnosis by thick film microscopy to confirm the accuracy of the RDTs for diagnosis under field conditions. The result of the RDT and the date of diagnosis were marked against the Household ID on the health facility ledger. Those found positive for malaria parasites were given artemether-lumefantrine (ALu), the first-line drug for treatment of malaria in Tanzania. Only participants that were RDT or slide positive for malaria parasites were treated. This was to avoid treating non-malaria patients with ALu, which might have affected malaria incidence rate in the village. The RDT's were labelled with the patient's unique identifier, date and status (+ve or -ve) and stored for verification. These were later checked against the clinical trial database to ensure that no cases had been incorrectly entered into the database by the clinic staff.

3.4 Data management

Data from the structured questionnaires on SES of households and KAP in relation to malaria and repellents administered at baseline; follow-up data on compliance and recalled febrile illness administered throughout the study period; and the after study KAP survey, were double entered into a computer using an Epi -Info™ template with a drop down lists of values that corresponded to the format of the questionnaires. Data was then exported to Microsoft Access 2008 (Microsoft Corporation), to check for lack/excesses of data, inconsistencies and outliers. All data from the above mentioned questionnaires were linked using the household unique identifier. The household unique identifier was made up of the household number, cluster number and treatment number.

3.5 Statistical analysis

Data was collected and presented at household and cluster level as the study aimed at assessing the effectiveness of the repellents at the community level. Individual level data was not collected.

3.5.1 Socio-economic status (SES)

All data cleaning and analysis was performed using STATA 11.2 software (StataCorp LP, College Station, Texas, USA). Baseline household-level socio-economic indicators were collected using a structured questionnaire. All variables representing asset ownership, household construction materials, source of fuel and light and the education level of the household head were examined individually before being combined using principal component analysis (PCA) to generate the socio-economic index of each household, [33], and are presented in here: (Appendix 1:Stata output showing Eigen scores of each variable used in calculation of socio economic status of households). The households were grouped into quintiles of the socio-economic index generated and ranked from the poorest to the least poor. This data was cross tabulated with treatment group using Pearson's chi-square (χ^2) to assess whether there was a significant difference in the socio-economic status of the households in the two treatment groups (not accounting for the clustered design due to the exploratory nature of this analysis).

The number of treatment tubes issued was analyzed by linear regression against month, treatment and an interaction of month and treatment to determine if there was a significant difference in the number of tubes issued in each month and per treatment group.

3.5.2 Clinical data

Clinical data was adjusted for covariates identified *a priori* to be confounders and analyzed using the according-to-protocol approach, where person-time at risk was excluded when a participant reported or was observed to be non-compliant to the lotion (placebo or repellent) and for those with malaria for three weeks after they were diagnosed. The total number of cases in each treatment group was divided by the sum of person years at risk to give the incidence rates in person years at risk. Rate ratio and rate differences were then estimated.

For comparison, a secondary analysis using the intention-to-treat approach, where malaria incidence rates in the clusters were compared using all person-time at risk regardless of whether they complied with the study protocol but also adjusted for covariates identified *a priori* as confounders. Such an approach would be expected to underestimate the treatment effect. It was not possible to effectively blind the PI and PL as they had carried out both the semi field and field efficacy evaluations of these treatments [19] and could identify the intervention and placebo. The clinical data was therefore re-blinded by an independent statistician (ET), who was not aware of the intervention and placebo codes.

3.5.3 Person-time at risk estimation for according-to-protocol analysis

The study was conducted for 14 months from July 2009 to August 2010. To calculate the person-time at risk, a closed cohort was assumed, so that the number of household members above six months recorded at baseline for each household was assumed to be constant throughout the study period. Monitoring surveys were conducted for each month of the study to establish compliance.

Person time at risk of each household was estimated according to one of the following three possible scenarios:

1. In a case where all individuals were susceptible to malaria infection and complied with the study protocol by applying the treatment issued on a nightly basis, each individual in the household was assumed to contribute one-person month at risk to the study.
2. In a case where the household head or an able household member was not available to take the monthly monitoring surveys, it was assumed that all members of that household did not comply with lotion (repellent or placebo) use for that month and one-person month at risk for each member of that household was excluded from the person time at risk of the study.
3. In a case where a household member contracted malaria, that individual was excluded from calculation of person time at risk for three weeks.

Person-time for all household members was calculated according to the appropriate scenario above.

3.5.4 Malaria incidence rates and regression analysis of the intervention effect

Using data on the total number of confirmed malaria cases and person-time for each household, we used a two-stage approach to estimate intervention effects (recommended by Hayes *et al.* for studies with fewer than 15 clusters/group) [27]. In the first stage, cluster-specific incidence rates were calculated using random effects Poisson regression modelling with adjustment for confounding variables. Specifically, the outcome of total number of confirmed cases of malaria/household was regressed on the set of confounding variables (age categories of the household, education of the household head, and quintile of SES), with an offset for person-time at risk per

household and a random intercept for cluster to account for the clustered study design. As per Hayes *et al.*, treatment was not included as a factor in the model. In the second-stage, residuals, calculated from the regression model were aggregated by clusters. The covariate-adjusted treatment effect was then estimated by comparing the residuals in the intervention relative to the control group using the Wilcoxon rank sum test, because the data were not normal.

3.5.5 Knowledge attitude and practice (KAP) of community members in relation to malaria and repellent

Baseline data on knowledge of malaria and malaria prevention practices and knowledge and practice in relation to repellents were analyzed using descriptive statistics in STATA 11.2 to assess whether there was an imbalance between the treatment arms. Data that recorded attitude with regards to repellents, perceived effectiveness and willingness to continue use and pay were also analyzed and these results are presented elsewhere.

3.6 Ethical and safety considerations

During recruitment, the household head was asked for written informed consent for themselves and all household members. If consent was obtained, all members of the household were recruited into the study. Study participants were free to withdraw from the trial at any time. All households in the village were issued with an LLIN for every sleeping space to ensure equity. All individuals from the study village were allowed free consultation, treatment and drugs (ALu) from the village dispensary at project cost. Participant confidentiality was maintained by using generated unique identifiers instead of individual names during analysis.

Participants were educated on correct repellent use and application. Children under 6 months were excluded from the trial. An illustrated label giving instructions in the native language (Swahili) on safe repellent use was provided on each tube. DEET repellent used in this study has undergone extensive toxicological tests and has been endorsed as safe for human use [32]. The concentration of DEET (15%), used in this trial was approved by the Tanzanian Pesticides Research Institute, the Tanzanian Bureau of Standards and is available in Tanzanian shops. Guardians to children < six months were reminded to put their children under an LLIN early to prevent them contracting malaria. A clinical officer (CO) was employed at the village dispensary by the project to perform RDTs and to investigate and treat any adverse effects arising from repellent use.

Ethical approval for the study was obtained from Ifakara Health Institute (IHI) (IHRDC IRB A46), Tanzanian National Institute of Medical Research (NIMR/HQ/R8a/VOL IX/780) and the London School of Hygiene and Tropical Medicine Ethical Review Board (LSHTM ERB 5174). IHI provided study monitoring.

3.7 Results

3.7.1 Trial profile and baseline data

The trial profile is summarized in Figure 3:2. In the intervention group 2,224 individuals were enrolled and 2,202 in the placebo group. Loss-to-follow up was higher in the placebo group: $n = 34$ versus $n = 16$, and no individuals withdrew from the trial. Similar numbers of person-years were analyzed: 1952.81 in the intervention group and 1972.38 in the control group of the trial. Baseline household level socio-economic data on education and gender of household head, age-groups of all study

participants, household construction material, source of cooking fuel and lighting and asset ownership were examined individually and are presented in Table 3.1. The gender of the household heads was comparable between the two treatment groups, with 55.33% (n = 514) females and 44.67 (n = 415) males. Most of the household heads had received some form of formal education, 82.81% (n = 702) while only 17.18% (n = 161) had no formal education. Of all participants recruited in the study, 17.55% (n = 771) were children under five years of age, 34.37% (n = 1,510) were between five to 18 years of age and 48.08% (n = 2,112) were above 18 years of age and age-category distribution was similar in the two treatment groups. The predominant source of energy used by the households was wood fire, 89.96% (n = 883), while the predominant source of lighting used was the traditional lamp, 93.76% (n = 871). Assessment of household construction materials demonstrated that most households in the study area had floors made from mud, 82.78% (n = 769), while tin and thatch were used equally as roofing materials, 49.35% (n = 457). Also, most households in the study area had walls made from bricks, 79.87% (n = 742). Socio-economic indices generated from PCA suggested an imbalance between the two treatment groups, with the control group demonstrating a higher SES than the intervention group, (Pearson's $\chi^2 = 17.5519$, $p = 0.002$), (Table 3:2).

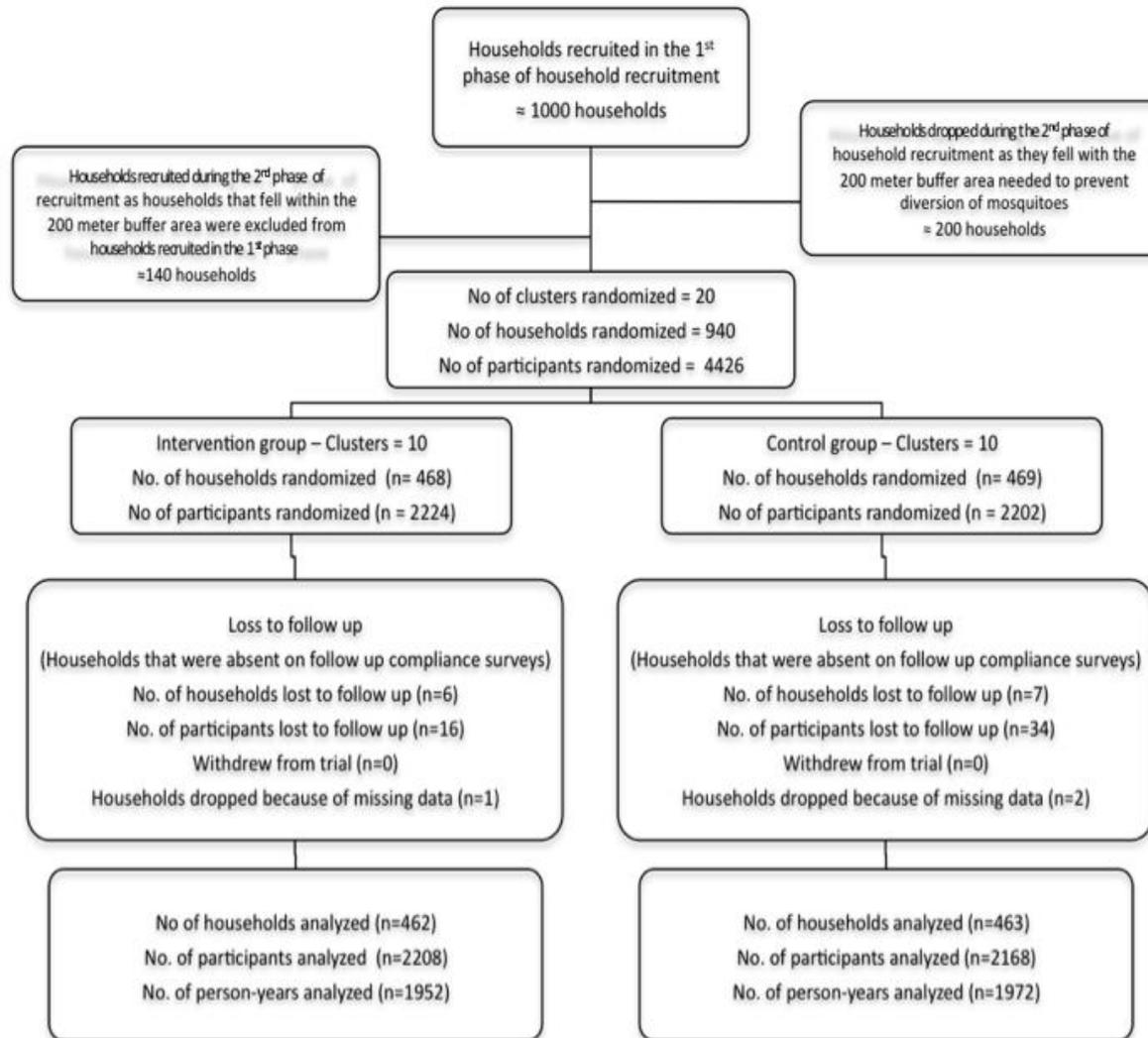


Figure 3:2 Trial profile

Table 3:1 Baseline household characteristics by treatment group

	Intervention arm n (%)	Control arm n (%)	Totals n (%)
No. of households	469(50.05)	468(49.95)	937(100)
No. of participants	2224(50.05)	2202(49.95)	4426(100)
<i>Gender of household head</i>			
Male	215 (46.24)	200 (43.10)	415 (44.67)
Female	250 (53.76)	264 (56.90)	514 (55.33)
<i>Education of household head</i>			
No education	83 (17.74)	78 (16.63)	161 (17.18)
Educated	385 (82.26)	391 (83.37)	702 (82.82)
<i>Age group distribution of all participant/household</i>			
Under 5's	412 (18.50)	359 (16.57)	771 (17.55)
5-18 years	721 (32.38)	789 (36.43)	1510 (34.37)
Above 18 years	1094 (49.12)	1018 (47.00)	2112 (48.08)
<i>Source of energy</i>			
Wood fire	431 (92.89)	402 (86.83)	883 (89.86)
Other sources	33 (7.11)	61 (13.17)	94 (10.14)
<i>Source of lighting</i>			
Traditional lamp	445 (95.70)	426 (91.81)	871(93.76)
Other source	20 (4.30)	38 (8.19)	58 (6.24)
<i>Flooring material</i>			
Mud	404 (86.88)	365 (78.66)	769 (82.78)
Cement	61 (13.12)	99 (21.34)	160 (17.22)
<i>Roofing materials</i>			
Thatch	256 (55.41)	201 (43.32)	457(49.35)
Tin	203 (43.94)	254 (54.74)	457(49.35)
Other	3 (0.65)	9 (1.94)	12 (1.30)
<i>Wall materials</i>			
Mud	121 (26.08)	66 (14.19)	187 (20.13)
Bricks	343 (73.92)	399 (85.81)	742 (79.87)
<i>Assets ownership</i>			
Motorbike			
Yes	72 (15.48)	52 (11.18)	124 (13.33)
No	393 (84.52)	413 (88.82)	806 (86.67)
Bicycle			
Yes	246 (52.90)	198 (42.58)	513 (55.16)
No	219 (47.10)	267 (57.42)	417 (44.84)
Stove			
Yes	344 (73.98)	314 (67.53)	658 (70.75)
No	121 (26.02)	151 (32.47)	272 (29.25)
Mobile phone			
Yes	197 (42.37)	211 (45.38)	408 (43.87)
No	268 (57.63)	254 (54.62)	522 (56.13)
Radio			
Yes	140 (30.11)	156 (33.55)	296 (31.83)
No	325 (69.89)	309 (66.45)	634 (68.17)

The use of repellents as a mosquito control tool was low in the study area, with only 1% (n = 6) of those interviewed reporting to have ever used repellents. Results on KAP of repellents are presented in detail elsewhere.

The average number of tubes issued per household was 6.73 (95% C.I. 6.51 – 6.95) and 6.92 (95% C.I. 6.68 – 7.16) in the intervention and control groups respectively and there was no significant difference per treatment group, 1.68 (95% C.I. 0.32 – 84.25, P = 0.803) from linear regression analysis. Likewise there was no significant difference on the number of treatment tubes issued per month throughout the study period.

Table 3:2 Ranking of households using Socio-economic scores generated for PCA analysis by treatment group

	Intervention arm n (%)	Control arm n (%)	Total n (%)	Pearson's Chi2	P value
<i>SES generated from PCA</i>					
Poorest	39 (8.33)	28 (5.97)	67 (7.15)	17.5519	0.002
Poor	164 (35.04)	121 (25.80)	285 (30.42)		
Median	165 (35.26)	174 (37.10)	339 (36.18)		
Less poor	77 (16.45)	107 (22.81)	184 (19.64)		
Least poor	23 (4.91)	39 (8.32)	62 (6.62)		

3.7.2 Clinical outcomes

3.7.2.1 *According-to-protocol analysis*

When data was analyzed as per protocol, there was a non-significant difference in cluster and household malaria incidence rates among repellent users and non-users (Table 3:3). In the cluster-level analysis (data averaged over cluster specific rates), the malaria incidence rates differed by 11.48%; with 68.29 (95% C.I 37.05-99.53) cases/1,000 person-years in the control group and 60.45 (95% C.I 48.30-72.60) cases /1,000 person-years (95% C.I. 44.55 – 81.73) in intervention group, (Wilcoxon rank sum $z = 0.529$, $p = 0.5967$). For household-level malaria incidence rates (data averaged separately over household specific rates), the incidence rates differed by 28.88%: with 84.54 (95% C.I 61.04-108.05), cases/1,000 person-years in the control group and 60.12 (95% C.I 45.08-75.15) cases/1,000 person-years in the intervention group, (Wilcoxon rank sum $z = -1.267$, $p = 0.2051$). These result should however be interpreted with caution as there is still an ongoing debate on whether it is correct to estimate incidence rate ratios using regression models on less than 10 clusters [28]. Cluster aggregated rates were reported because it measured the overall effect of the intervention at the population level [34] and this was the major objective of the study. Age was a significant risk factor with risk decreasing with increase in age. SES did not influence the risk of malaria in the model.

Table 3:3 Estimated incidence rate by treatment arm and estimated intervention effects

	Intervention arm	Control arm	% Reduction in rates	Wilcoxon rank-sum on residuals (p-value)
Malaria cases	115	137		
ATP analysis				
Individuals randomized	2208	2168		
Households randomized	463	462		
Total person-years	1952.81	1972.38		
Average Household rates/1000 person-years	60.12 (95% C.I 45.08-75.15)	84.54 (95% C.I 61.04-108.05)	24.42%	-1.267 (0.2051)
S.D.	164.42	257.07		
Average cluster rates/1000 person-years	60.45 (95% C.I 48.30-72.60)	68.29 (95% C.I 37.05-99.53)	8%	0.529 (0.596)
S.D.	16.98	43.66		
ITT analysis				
Individuals randomized	2224	2202		
Households randomized	468	469		
Total person-years	2580.44	2554.92		
Household rates/1000 person-years	47.26 (95% C.I. 35.49-59.04)	68.21 (95% C.I. 49.59-86.84)	20.95%	-1.268 (0.2047)
S.D.	129.60	205.23		
Cluster rates/1000 person months	45.43 (95% C.I 36.02-59.79)	53.21 (95% C.I. 30.98-104.16)	7.78%	0.227(0.8206)
S.D.	11.32	34.90		

3.7.2.2 Intention-to-treat analysis

Cluster-level analysis of malaria rates in the two treatment arms demonstrated a non-significant, 14.62% difference in malaria rates with 53.21 cases/1,000 person-years (95% C.I. 30.98 – 104.16) in the control group and 45.43 cases /1,000 person-years (95% C.I 36.02 – 59.79) in the intervention group, (Wilcoxon rank sum $z = 0.227$, $p = 0.8206$), (Table 3:3). Household-level analysis of malaria incidence rates demonstrated a 30.71% difference in malaria incidence rates, with 68.21 cases/1,000

person-years (95% C.I. 49.59 to 86.84) in the control group and 47.26 cases/1,000 person-years (95% C.I. 35.49 – 59.04), in the intervention group, (Wilcoxon rank sum $z = -1.268$, $p = 0.2047$). Age was a significant risk factor: malaria risk decreased with increase in age although SES did not influence the risk of malaria in the model.

3.8 Discussion

This randomized controlled trial demonstrated that 15% DEET topical repellents have no effect on malaria incidence transmitted in the early evening. Although there was a consistent decrease in malaria risk among repellent users in both the cluster and household malaria rates, as seen from the results above, this reduction was not significant. This finding is consistent with a study carried out in southern Lao PDR using an identical 15% DEET repellent [35]. It should be noted that, findings from other studies using a higher concentration of 20% DEET with Permethrin in soap that gave over 12 hours of complete protection from mosquito bites [11] and Para-menthane 3–8 diol repellents with close to 100% efficacy for over six hours [30,36] did demonstrate a significant protective effect in Pakistan [11], Bolivia [10] and Ghana [23] and this could be one of the potential explanations for the observation of a treatment effect in these studies. It can be argued that in the Lao-PDR study, 15% DEET provided ~ 100% protection against mosquito bites. However, the number of major malaria vectors, *Anopheles minimus* and *Anopheles maculatus*, caught in entomological collections in the Lao-PDR study was very low and that the effect observed, was probably that of 15% DEET against *Stegomyia* and *Culex* mosquitoes which made up the bulk of the collections. Therefore, as Anophelines are known to show less response to repellents compared to *Stegomyia* and *Culex* mosquitoes

[37,38], the repellent effect observed in the Lao-PDR study was greater than at higher densities with a greater proportion of Anophelines as tested in Tanzania [19].

3.8.1 Power

There are several factors that are likely to have masked the treatment effect in this study, the most likely being the lack of power to discriminate a statistically significant difference between study arms. The lack of power in the study was likely caused by four factors:

First, rapid scale-up of LLINs to achieve universal coverage has been actively taking place in Tanzania [16]. This had led to a substantial decline in malaria in the country and by extension the study area [39]. As a result, the incidence of malaria in the village was likely lower than the incidence assumed for calculation of sample size for this study. This likely led to an underestimation of the sample size required to observe a difference between the two treatment groups. Secondly, during the study period, Tanzania experienced a drought that likely further reduced malaria transmission, and as a result, there were too few malaria episodes in the study area to accurately discriminate any reduction in malaria attributable to the repellent [40], highlighting the need for such studies to be carried out for more than one transmission season to avoid such problems. Third, most of the participants recruited in to the study come from a farming community. Therefore, during the planting and harvesting seasons, these participants relocated to their farmhouses [41]. As a result it was difficult to establish compliance during these periods and those participants were excluded from the study. This lowered the study sample size further and with it the power to detect a treatment effect. Lastly, there was the likely overestimation of the assumed malaria incidence in the study area that was used for sample size calculations. Malaria

incidence in this study was estimated from reported fever rates in children less than 5 years of age in the study area [26]. Therefore, even though scale up of LLINs and the drought experienced during the study might have lowered the malaria incidence in the study area, it is also likely malaria rates used for estimation of sample size might have been overestimated and hence undermined the study power to observe a difference between the treatment groups.

3.8.2 Compliance

Compliance in this study was measured by self-reporting of use every evening by the household head or a household member that was able to engage with the field workers during the monitoring surveys. However self-reporting is an unreliable measure of compliance, as it has been shown to overestimate compliance [42]. As a result, the ATP analysis used to measure malaria incidence is likely to underestimate the actual malaria incidence in the intervention and control arms, as a larger value of person-time will be used than that of individuals that actually complied to the study reducing discriminatory power. However, if the randomization between the two treatment groups was done correctly then the overestimation of compliance and its resultant effect on the study outcome, is likely to be similar in both treatment groups, ruling out the likelihood of overestimation of the treatment effect. This underlines the importance of correctly estimating the compliance in studies of personal protection in order to avoid confounding the outcomes of such studies.

3.8.3 Active versus passive case detection

Due to logistical reasons, this study recruited a single government health facility for collection of clinical data by passive case detection. As a result, the study is likely to have lost malaria cases to the other health facility present in the area. Anecdotally,

some participants complained that they went to the other health facility because the study facility always told them that they did not have malaria even though they *knew* they had malaria, so they did not trust the diagnosis. Also some individuals might have opted to use traditional medicine, treat diseases at home or buy drugs directly from the numerous drug stores in the study area if they felt sick. All these are potential malaria cases that the study might have lost, lowering both the sample size and estimates of malaria incidence in the area. It would have been advantageous to collect data from both health facilities or carry out active case detection. Since malaria was still most common in children under five years in the study site as seen elsewhere [43,44], targeted active case detection in under fives may have gathered more reliable and realistic data on the true impact of repellents in this scenario. Performing supplementary testing of blood spots from all participants attending the health facility with polymerase chain reaction (PCR) diagnosis of subclinical malaria parasitaemia may have also yielded more accurate estimation of transmission prevention by repellents [45].

3.8.4 Sources of bias

Bias was introduced into the study by an imbalance in socio-economic status between the two study groups. The control group demonstrated a higher socio economic status than the control arm. This study did not demonstrate a statistically significant association between SES and malaria incidence. However, it is well known that improved housing, whose representative covariates had been adjusted for during analysis, is protective against malaria [46]. A plausible explanation for this is that the participants in this study came from a single village or from villages located closely together. As a result, they were exposed to the same levels of malaria transmission regardless of their socio-economic status. As socio-economic status is positively

associated with seeking treatment at a medical facility [47], it is likely that participants with higher SES sought treatment at the health facility in the study area at a higher rate compared to participants in the lower SES. Therefore as malaria data was only collected from a single health facility, it is likely that more cases of malaria were observed in participants with higher SES relative to participants from lower SES. Another reason that no association was seen may be because studies using material ownership as a proxy for measuring SES, to evaluate the relationship between SES and malaria incidence have yielded inconsistent results, at the household level [48].

The study participants were blinded up to some point after allocation of treatments, because of the identical packaging labelled with a three-digit code. However, after a while, field workers reported that study participants in the placebo group complained that they wanted to swap treatment. Participants could differentiate the intervention from the placebo, as mosquitoes would still bite them after applying the ‘treatment’ while those in the treatment group bragged to their neighbours that they got the good lotion that was effective. This is a source of bias and could have caused treatment contamination between clusters. This problem would have been better overcome with clusters that were geographically isolated, for instance randomization on a village scale, so that individuals were less likely to be able to compare their treatment allocation. Some participants may have sold or given their repellent to relatives in other clusters.

Another potential confounder may have been diversion of mosquitoes from the intervention group to the placebo group. However, this was controlled by allowing for a buffer area of 200 metres between clusters. Diversion in repellent studies has

usually been recorded over short distances, (one meter, 1m) [30]. However, distances of 15–20 metres are recommended as the limit for short range attraction of host seeking mosquitoes [49,50] and, therefore, distances of 200 metres between clusters were thought to be adequate to prevent diversion. Treatments were also issued at the household level to prevent intra and inter-household diversion within the cluster. It was later observed in the study area that mosquito diversion between households does occur [29] and could have confounded data if compliance with the intervention was low by diverting mosquitoes from complying to non-complying households or individuals.

The community was highly knowledgeable about malaria transmission, prevention and control. This is likely a result of the malaria intervention programmes that have taken place in the study village for over two decades [14,17]. The community awareness about topical repellents as a mosquito control tool was poor at the study inception. However, after the study, the community was highly aware of repellents and community members were willing to take up this intervention against malaria if available. This finding demonstrates the feasibility of topical repellents as a potential tool to supplement LLINs to prevent early evening transmission. In a separate study, the community members reported bite avoidance as the major reason for using repellents in the early evenings.

A *posteriori* analysis of data for children under six months was carried out to check whether this age group experienced higher malaria transmission because of mosquitoes diverted to them as it was recommended that they not use the repellent [29,30]. This might also have affected the incidence of malaria in the treatment groups if there was uneven distribution of this age category between these groups.

However, it was observed that there were only three children and a single case of malaria in this age category, and it can be confidently concluded that this age group did not have any influence on the outcomes observed.

Net usage was also analyzed to determine whether there was a difference between the two treatment groups, which would have confounded the outcome. It was observed that reported net usage the previous night was 100% in both treatment groups. These results are presented in detail elsewhere.

3.9 Recommendations

It was observed that estimation of a sample size with sufficient power was a major shortcoming of this study. Therefore, it is advisable to establish baseline disease incidence rates if a similar study is to be implemented in the future to avoid under powering the study. This can be established from health facility records. However these records may not necessarily be accurate and the more appropriate measure may be to conduct a small cross-sectional or longitudinal survey of the community disease prevalence or incidence and then power accordingly. Another important factor when testing personal protection tools is accurate establishment of compliance. Better methods of establishing compliance are needed. This can be done through frequent follow-up and spot checks or use of indirect methods, such as mosquito saliva antigens, that are a proxy of individual exposure to mosquito bites [51]. Also, development of new tools that require reduced compliance such as long lasting spatial repellents [52] would likely offer greater protection because people often forget to comply daily with a topical repellent unless they feel mosquito bites [53]. Finally, in a time when malaria is becoming more scant due to successful control, active case detection using RDT for clinical diagnosis followed up by PCR for malaria parasites

is most likely the most appropriate means of measuring the impact of additional malaria control tools used in combination with LLINs.

3.10 Conclusion

Findings of this trial could not demonstrate if 15% DEET topical repellents had any impact on incidence of malaria transmission in the early evening because the study lacked sufficient statistical power and had several important sources of bias. A better-designed study with sufficient power and fewer sources of bias and ideally a higher concentration of repellent is required to fully understand if topical mosquito repellents are a feasible malaria control tool in the early evenings in Eastern Africa, particularly as repellents have reduced malaria transmission elsewhere in sub-Saharan Africa [23,24]. The acceptability of this intervention is an encouraging finding toward exploring supplementary malaria control tools.

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Chapter 4: Feasibility of repellent use in a context of increasing outdoor transmission: a qualitative study in rural Tanzania



4.1 Abstract

Background

Extensive employment of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) has substantially reduced malaria morbidity and mortality in sub-Saharan Africa. These tools target indoor resting and biting vectors, and may select for vectors that bite and rest outdoors. Thus, to significantly impact this residual malaria transmission outdoors, tools targeting outdoor transmission are required. Repellents, used for personal protection, offer one solution. However, the effectiveness of this method hinges upon its community acceptability. This study assessed the feasibility of using repellents as a malaria prevention tool in Mbingu village, Ulanga, Southern Tanzania.

Methodology

Change in knowledge, attitude and practice (KAP) in relation to repellent use was assessed before and after the implementation of a cluster randomized clinical trial on topical repellents in rural Tanzania where repellent and placebo lotion were provided free of charge to 940 households for a period of 14 months between July 2009 and August 2010. Compliance, defined as the number of evenings that participants applied the recommended dose of repellent every month during the study period, was assessed using questionnaires, administered monthly during follow up of participants in the clinical trial. Focus group discussions (FGDs) were conducted in the same community three years later to assess the community's KAP in relation to repellents and preference to different repellent formats.

Results

At baseline, only 0.32% (n = 2) households in the intervention arm and no households in the control arm had ever used topical repellents. During follow-up surveys, significantly more households, 100% (n = 457) in intervention arm relative to the control, 84.03% (n = 379), ($p = <0.001$) perceived the repellent to be effective.

Post-study, 99.78% (n = 462) and 99.78% (n = 463), ($p = 0.999$) in the intervention and control arms respectively, were willing to continue repellent use. Mosquito nuisance motivated repellent use. From the FGDs, it emerged that most respondents preferred bed nets to repellents because of their longevity and cost effectiveness.

Conclusion

High repellent acceptability indicates their feasibility for malaria control in this community. However, to improve the community's uptake of repellents for use complimentary to LLINs for early evening and outdoor protection from mosquito bites, longer lasting and cheap formats are required.

Keywords

Repellent, Malaria, Knowledge, Attitude, Perceptions, Practice

4.1 Background

Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) have had a great impact on malaria morbidity and mortality in the past decade in sub-Saharan Africa [1-3]. While effective, these tools are intra-domiciliary and predominantly target indoor biting and resting vectors [4]. This favours outdoor resting and biting vectors as IRS and LLINs are less effective against those vectors that exhibit exophily and exophagy [5]. Therefore, as malaria moves from sustained control to elimination, new tools that tackle residual outdoor malaria transmission are needed.

Repellents used outdoors and in the early evenings and mornings, where IRS and LLINs cannot be employed, present one strategy that can be used to push towards the goal of eradication. Topical (skin applied) repellents have been used as a form of personal protection for hundreds of years [6], and have been shown to protect against malaria in South America (80% reduction) [7] and Southern Asia (60% reduction) [8], and more recently in Ghana (34% reduction) [9] and Ethiopia (19% reduction) [10]. The major drawback to using topical repellents is compliance. Topical repellent use requires daily use and frequent re-application as their effect is usually short-lived over a few hours and therewith a change in daily routine (personal behaviour). While changing personal behaviour to use new interventions is not impossible as has been demonstrated in bed net campaigns [11], oral hygiene [12] and hand washing strategies [13], it is influenced by a number of other factors including: cost, perceived quality of the intervention, accessibility, information and ease of use. An intervention is likely to be used by the community if its affordable, perceived to be effective, the community is aware and has knowledge of its uses and finally, the intervention is simple to apply, i.e. it does not require considerable deviation from daily routine [14].

Therefore to influence behaviour change towards uptake of interventions: the community must be educated to improve information on the appropriate measures to employ to prevent disease e.g. use of bed nets to prevent mosquito bites and hence malaria infection. Secondly, the interventions must be made physically accessible to the community, such as considering the distance to shops where bed nets are sold or re-treated. Third, the cost of the intervention must be affordable and perceived as reasonable among community members to encourage use. Perception of the effectiveness of the intervention will also influence uptake, with the community more likely to use interventions they perceive as beneficial to them, for instance LLINs prevent mosquito bites. Lastly is the ease of use of the intervention being implemented, as the community is more likely to use interventions that require the least deviation from daily routine, like use of drugs with simple dose regimens compared to those that have complicated regimens [14].

Therefore, in an effort to determine the feasibility of using repellents as a mosquito control tool, this study assessed the knowledge/awareness, acceptability, perceptions on effectiveness and preference to different kinds of repellents in a rural community in Kilombero valley, Southwest of Tanzania. The community in this setting has experienced extensive malaria research projects and intervention programmes spanning two decades [15-17] and was expected to be highly knowledgeable about malaria prevention and control. Cooking mainly takes place outdoors and in the early evening, a situation that exposes the community to nuisance mosquito bites and potential malaria transmission before they have employed bed nets. Further, like the rest of sub-Saharan Africa, the study area is experiencing rapid rural development, shifting the spaces and protocols of social behavior. Where once it was customary to retire shortly after sundown, now, owing to rural electrification programmes, residents

usually gather in the early evening and stay late into the night at local bars and social centers springing up in the study area, thereby increasing perception of mosquito nuisance and malaria transmission potential at these times.

The dominant vector in this area is *Anopheles arabiensis* [18] that has been shown to shift to early evening and outdoor biting when hosts are unavailable late in the night indoors as a result of high bed net use [19,20]. The presence of rice fields in the study area, as the community's main occupation is farming, provides for a large breeding site of mosquitoes [21]. The presence of this large breeding site is likely increase mosquito abundance in the study area, and with it potential malaria transmission and nuisance biting.

Before the start of the clinical trial, the community was sensitized to the potential for repellents as a malaria prevention tool through skits, community meetings and leaflets. Therefore, they are likely to understand the importance of topical repellents in prevention of early evening malaria transmission potentially occurring in the study area before they go to sleep under bed nets, and are therefore more likely to be receptive to this intervention. Secondly, the customary practice of cooking outdoors as well as presence of electricity exposes this community to nuisance biting in the evenings as a result of the extensive rice fields present in the area, a situation likely to encourage use of repellent. Finally, repellents were provided free so the community was likely to use them and form an opinion on their efficacy.

4.2 Methods

4.2.1 Study area and population

This study was conducted in Mbingu village, Kilombero district, Tanzania, situated 55kms west of Ifakara town at 8.195°S and 36.259°E. There is malaria transmission all year round, with peak transmission occurring in the months of May and June after the long rains. The village experiences an annual rainfall of approximately 1,200-1,800 mm and an annual temperature range of between 20°C and 32.6°C. The village borders an extensive field cleared for irrigation, which provides an ideal breeding site for malaria vectors. The houses in the village are clustered in groups of 3–5 households, which mainly belong to one family, but in a few instances the houses may be rented by different families. In July 2009 (at the inception of the clinical trial), the population of the study area was estimated to be 7, 609, with each household having approximately 5 members [22]. Most houses are constructed from mud walls and thatched roof, with one-third made from brick walls and corrugated iron roof.

4.2.2 Outline of study

Between July 2009 and August 2010, a placebo-controlled cluster randomized clinical trial was conducted in the study village where 15% DEET (*N, N*-Diethyl-3-methylbenzamide) topical repellent and an identical placebo lotion were randomly issued to 940 households in the study village [23]. The clinical trial participants were also issued with double size LLINs per sleeping space to ensure equity. Treatments were issued to two study arms of 10 clusters with 47 households each. One study arm was issued with topical repellent lotion while the other study arm received a placebo lotion and both arms were followed up for 14 months to assess the malaria incidence between these two groups. Concurrent with the clinical trial, a knowledge, attitude

and practice survey (KAP) of the repellents issued during the clinical trial was conducted by administering a questionnaire (Appendix 2: Repellent KAP survey tool) at the baseline of the clinical trial (before/entry survey) to assess community knowledge of repellents; at the beginning of every month when field workers visited the households to replace repellents that had run out (follow-up survey) throughout the study period, to assess the acceptance and compliance of the community to the repellent issued and perceived effectiveness; and at the end of the clinical trial (after/exit survey) to assess willingness to continue use of repellents. This tool was designed such that respondent were allowed a single answers to multiple questions like; e.g.: why did you not use repellents last night and were expected to pick one answer from multiple choices outlined (Appendix 2: Repellent KAP survey tool). It should also be noted that there are several questions that might have been subject to Hawthorne's effect, where the respondent's likely answered questions according to what they thought the investigators desired to hear. These questions are; Perceived effectiveness, compliance and whether they like repellents or not (Table 4:1, 4:2 & 4:3). A separate Focus Groups study was conducted three years later in June 2013.

4.2.3 Baseline survey

At baseline, written informed consent was sought from the household heads that were willing to participate in the clinical trial. The household heads gave consent for all household members who were below 18 years. Household members above 18 years were asked to sign their own written consent forms. As the household was analyzed as a unit, a structured questionnaire of KAP in relation to repellents was administered to the household head. A unique ID was stapled on the door of each household that was recruited into the study.

4.2.4 Follow-up survey

To assess acceptability and use, at the beginning of every month after the baseline survey, field workers visited the households recruited in the study to replace the tubes of repellent issued the previous month. A KAP questionnaire was administered during these visits, where the households were asked if they liked the repellent issued and their perceptions on the effectiveness of the repellent. The fieldworkers also administered a compliance questionnaire, where household members were asked if any household member had skipped a day of repellent use in the past month and reasons for missing that day. However, if during the follow up survey there were no household members present to answer the questionnaire on compliance, and continued to be absent for seven consecutive days after the first visit to assess compliance, that household was considered non-compliant to repellent use for that month. If the households reported that any household member did not use the repellent, that household member was removed from follow up time for the period they did not use the repellent. Thus, if all household members reported using repellent each night in the past week and an adult member of the household was present to be issued with new repellent, that household was considered compliant for the previous month. In addition, the number of treatment tubes (repellent and placebo tubes) issued per month was recorded, to determine if there was a difference in the number of tubes issued in each month per treatment group. Differences between recalled and observed compliance were not measured.

4.2.5 Post-study survey

At the end of the clinical trial, (August 2010), an exit KAP (post-study) questionnaire to assess perceptions on effectiveness and willingness to pay if repellent was provided at cost was administered. In particular, the respondents were asked what was their

perceived cost for the repellent issued during the clinical trial. They were also asked how much they were willing to pay for the tube of repellent they were given during the clinical trial.

4.2.6 Focus group discussions

4.2.6.1 In-depth discussions

Seeking an in-depth understanding of the knowledge, attitude, perceptions and practice in relation to repellents as a vector control intervention, a descriptive exploratory study, consisting of seven Focus-Group-Discussions (FGDs) and one Small Group Interview (SGI) was conducted in the study village from 10th – 28th June 2013, three years after the clinical trial. The participants may or may not have participated in the initial clinical study of topical repellents, as prior participation in the previous trial was not an inclusion or exclusion criterion. Several different formats of repellents were provided to participants to measure perceived preferences in delivery formats of repellents among members of a community that had previous familiarity with repellents.

4.2.6.2 Sampling of FGD participants

This study initially used convenient sampling to enroll household heads in the village. A purposive sample of households with the following characteristic were drawn from the community:

- Households that had the males as household heads.
- Households that had females as household heads (widows, divorced, separated etc.).

- Households that had males as household heads but from which their female partners were invited for the FGDs and SGI.
- Households that had children of school going age (both primary and secondary schools).

From this sample, 6 – 12 individuals from households with each of the above characteristics were interviewed in seven FGDs and one 5-member SGI. The FGDs were dynamic in nature consisting of individuals from 10 to 60 years of age and sampling was stopped at the ‘point of saturation’ (no further ‘new’ information generated).

4.2.6.3 Study tools

Based on literature on knowledge and practice in relation to repellent use and on *a priori* experience of repellent work with the community in the study area, an interview guide on perceptions and practices around repellent use in Mbingu village was developed for conducting the FGDs. This guide was pre-tested on four villagers, two men and two women before undergoing further changes based on the feedback from these villagers. The outcome was a simple interview guide that consisted of six open ended questions that were structured in a flexible manner to allow for any emerging ideas from the participants to be incorporated there in.

4.2.6.4 Repellents explored

The different types of repellents issued to the participants of this study were; Permethrin impregnated ‘*kangas*’ (a sheet of fabric worn around the waist by women in Africa), 15% DEET (*N, N*-Diethyl-3-methylbenzamide) topical repellent in petroleum jelly format, 15% DEET topical repellent in spray format, 30% PMD

(Para-Methane 3-8-diol) topical repellent in lotion format, 30% PMD topical repellent in spray format, 2% transfluthrin impregnated sisal strip (sack), that was hung in a common area where all household members sat, (Figure 4:1) and 2% permethrin impregnated net fencing that was designed to protect individuals sitting outdoors, especially around the cooking area (Figure 4:2).



Figure 4:1 Testing the efficacy of transfluthrin impregnated sisal strip in the semi-field system at the IHI



Figure 4:2 Installation of permethrin-impregnated fencing around an outdoor kitchen/cooking area in the study area

4.2.7 Procedures

Participants were verbally informed on the objectives and aims of the study, its voluntary nature, risks and benefits. Thereafter verbal informed consent was sought from the purposive and final sample of participants after all ethical considerations of the study had been outlined. Interview schedules, including convenient interview times and venues were then negotiated between the study investigators and participants and the study commenced from the 10th to 28th of June 2013. The interviews were all conducted in Swahili and lasted between 30mins and 1 hour in the various local settings preferred by the participants. Consent was sought to use a tape recording device for the sessions with all villagers agreeing to be tape recorded prior to commencement of the interviews. First, four FGDs with the four different respondent groups: households that had the males as household heads, households that had females as household heads (widows, divorced, separated etc.), households that had males as household heads but from which their female partners were invited for interviews, and households that had children of school going age (both primary and secondary schools), were conducted where community knowledge (familiarity) and use of repellents as a mosquito control tool was assessed. At the end of these first four FGDs, the respondent groups were issued with different formats of repellents to use for a week. After using the different repellent formats for one week, these respondents groups were recalled for a further three FGDs and a single SGI where experiences of repellent use and preference to different repellent formats were assessed.

4.3 Data management

Data from the baseline, follow-up, and post-study surveys were linked using the household unique identifier. Data from these questionnaires were entered into and coded using an Epi-info template that corresponded to the format of the questionnaires. All data was double entered into Epi-info, where it was checked for excesses or missing of data. Data was then exported to Microsoft Access 2010 database where it was checked for duplicates. Data from the FGDs was collected using tape recorders and imported into the computer where they were stored as audio files ready for transcription and analysis.

4.4 Data analysis

All data analysis was carried out in STATA 11.2 (StataCorp LP, College Station, Texas, USA) software. Data from the baseline, follow-up and post-study surveys were analyzed using descriptive statistics and are presented in tables (Tables 4:1, 4:2 & 4:3).

Data from the socio-economic status (SES) was analyzed using principal component analysis (PCA). A socio-economic index was generated using PCA and the generated score used to show wealth index of each household. Indicators of (SES) used were; asset ownership, household construction materials and education level of household head. These results are reported in detail elsewhere [23]. Data for KAP collected during the follow-up survey was analyzed by determining trend over time, using descriptive statistics. Compliance data collected using the follow-up survey was also stratified by SES quintiles to determine if there was a difference in repellents use by SES quintile.

Data for KAP collected at baseline and post-study survey was analyzed by comparing the before and after studies using descriptive statistics. Likewise, in the post-study survey, willingness to pay was compared across the SES quintiles.

The number of repellent and placebo tubes issued was analyzed by linear regression against month, treatment group and an interaction of month and treatment group to determine if there was a significant difference in the number of tubes issued in each month and per treatment group.

Data collected over the study period (follow-up survey) was used to report outcomes on compliance, community liking the repellent and perception of effectiveness of repellents because it was assumed to be less prone to recall bias compared to data collected at the end of the clinical trial (post-study survey).

Audio files from FGDs were transcribed verbatim in Microsoft Office and imported into Nvivo 9 (QSR international Pty Ltd 2006–2010) qualitative analysis software. The data was then coded into themes as they emerged from the response data in the transcripts. This content analysis also allowed for themes emerging from the data to be considered during iterative coding. The final coding tree (structure of categorizing data) consisted of identified themes from the data as well as unanticipated themes from the respondents. The final stage of the analysis involved re-organization of the themes into larger categories of themes communicating the key messages from each of the smaller themes under them (Table 4:4).

4.5 Ethical consideration

Participants were recruited on written informed consent. Ethical approval for the study was obtained from Ifakara Health Institute (IHI) (IHRDC IRB A46), Tanzanian National Institute of Medical Research (NIMR/HQ/R8a/VOL IX/780) and the London School of Hygiene and Tropical Medicine Ethical Review Board (LSHTM ERB 5174). IHI provided study monitoring.

4.6 Results

4.6.1 Baseline survey

At baseline, only 0.32% of the households had ever used repellents in the intervention arm, while no households had ever used repellents in the control arm (Table 4:1).

Table 4:1 Baseline perceptions on malaria and repellents

	Repellent n (%)	Placebo n (%)	Totals n (%)	P- value
<i>What is malaria</i>				
Disease	285 (93.44%)	270 (95.07%)	555 (94.23%)	0.397
Don't know	20 (6.56%)	14 (4.93%)	34 (5.77%)	
<i>Causes of malaria</i>				
Mosquitoes	302 (99.01%)	280 (98.59%)	582 (98.81%)	0.634
Other	3 (0.99%)	4 (1.41%)	7 (1.19%)	
<i>Knowledge of malaria prevention methods</i>				
Bed nets	286 (94.38%)	271 (95.42%)	557 (94.89%)	
Environmental management	7 (2.31%)	3 (1.05%)	10 (1.70%)	
Going to hospitals	4 (1.32%)	2 (0.70%)	6 (1.02%)	
Using repellents	1 (0.33%)	1 (0.35%)	2 (0.34%)	
Don't know	5 (1.65%)	7 (2.46%)	12 (2.04%)	
<i>Knowledge of mosquito breeding site</i>				
Water puddle	291 (95.40%)	270 (95.40%)	561 (95.41%)	0.998
Other	14 (4.60%)	13 (4.60%)	27 (4.59%)	
<i>Protection methods used</i>				
Bed nets	294 (95.14%)	277 (96.85%)	571 (95.97%)	0.600
Mosquito Coils	3 (0.97%)	3 (1.04%)	6 (1.01%)	
Environmental management	7 (2.26%)	5 (1.74%)	12 (2.02%)	
Covering oneself	4 (1.29%)	1 (0.34%)	6 (0.84%)	
Using repellents	1 (0.32%)	-	1 (0.17%)	
<i>Reasons for using protection methods</i>				
Effective	174 (56.31%)	154 (54.03%)	328 (55.22%)	0.008
Readily available	34 (11.00%)	22 (7.71%)	56 (9.34%)	
Cheap	23 (7.44%)	8 (2.80%)	31 (5.22%)	
Easy to use	76 (24.59%)	100 (35.08%)	176 (29.63%)	
Other	2 (0.64%)	1 (0.35%)	3 (0.51%)	
<i>Reasons for not using repellents</i>				
Don't understand use	139 (45.27%)	118 (41.40%)	257 (43.41%)	0.057
Not aware of repellents	38 (12.37%)	28 (9.82%)	66 (11.15%)	
Not available	109 (35.50%)	115 (40.35%)	224 (37.84%)	
Expensive	16 (5.21%)	24 (8.42%)	40 (6.76%)	
Other	5 (1.62%)	-	5 (0.84%)	
<i>Willingness to use repellents</i>				
Yes	309 (99.67%)	286 (100%)	595 (99.83%)	0.336

No	1 (0.32%)	-	1 (0.17%)
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Two households reported burning mosquito coils, five households repelled mosquitoes with a smoky fire and one household reported using repellent plants (data not shown). Most households (95.7%) used bed nets as these had been delivered through various governmental and non-governmental schemes from 1997 onwards. When asked about malaria a similar proportion of the households in the intervention and control arms reported that malaria is a disease: 93.44% (n = 285) and 95.50% (n = 284), respectively. When asked about malaria transmission, most households in the intervention arm 99.01% (n = 302) and control arm 98.59% (n = 280) reported that mosquitoes transmit malaria. Bed nets were the major prevention tool used in the study village, with a similar proportion of reported bed net use in the intervention 95.14% (n = 294) and control arm 96.85% (n = 277). When households that reported bed net use, were further asked why they preferred bed nets to other tools, a significantly larger proportion cited effectiveness relative to other reasons: 56.31% (n = 174) and 54.03% (n = 154) in the intervention and control arm, respectively. Other reasons for use of bed nets as well as other mosquito bite protection methods are reported in Table 4:1. It should be noted that the bed nets reported by the respondents, were not those issued during the clinical trial, but they were reporting on tools they used before the onset of the clinical trial. However, bed nets were given at the start of the clinical trial to ensure equity between the study arms. An equal proportion of households in both the intervention 95.40% (n = 291) and control 95.40% (n = 270), arms reported that mosquitoes breed in standing water. The major barrier to repellent use in this community was lack of knowledge on how to use repellents, with 45.27% (n = 139) households in the intervention and 41.40% (n = 118), in the control arm reporting that they did not understand how topical repellents were used. Lack of

awareness of repellents was also reported as a barrier to repellent use, with 35.50% (n = 109) and 40.35% (n = 115) of the households in the intervention and control arms respectively, indicating that they were not aware of repellents as a mosquito control tool.

However, when repellents were made available knowledge was no longer a barrier to compliance. All households were willing to use repellents to prevent mosquito bites: 99.67% (n = 309) of the households in the intervention and 100% (n = 286), (p = 0.336), in the control arm were willing to use repellents, even though this tool was novel in this community after community sensitization, (Figure 4:3).



Figure 4:3 Community sensitization meeting on repellents conducted by the social marketing team from IHI.

4.6.2 Follow-up survey

A follow up survey was conducted to assess household compliance to repellent use. Compliance in this context is defined as having recalled use of the repellent every night in the past month. However, if during the follow up survey there were no household members present to answer the questionnaire on compliance, and continued to be absent for seven consecutive days after the first visit to assess compliance, that household was considered non-compliant to repellent use for that month. If the households reported that any household member did not use the repellent, that household member was removed from follow up time for the period they did not use the repellent. Reported household compliance with repellent use was not significantly different between the study arms: 81.50% (n = 379) in the intervention and 78.13% (n = 361) in the control arm, (p = 0.202) during the study period. Significantly more households liked using the repellent in the intervention arm 99.35% (n = 462) compared to the control arm, 84.41% (n = 390), (p = <0.0001). When asked about effectiveness, significantly more households in the intervention arm, 100% (n = 465) compared to the control arm 84.03% (n = 379), (p = <0.0001), perceived repellents to be effective (Table 4:2). Also, significantly more households that perceived the repellent to be effective complied with repellent use (72.31%) compared to those households that did not comply (27.68%), (p = <0.0001). This indicates that relief from mosquito bites was a motivating factor in repellent compliance.

When the perceptions of effectiveness of repellents were analyzed over the study period, it was observed that there was an increase in the number of households reporting the repellent to be effective over time. This trend was also observed for households that reported to like the repellents. Compliance was observed to increase

over the study period, with more households reporting repellent use at the end of the study compared to the start of the study. Because the repellents were given out for free there was no difference in repellent compliance between the most poor and least poor socioeconomic quintiles ($p = 0.369$), data not shown.

There average number of tubes issued per household was 6.73 (95% C.I. 6.51 – 6.95) and 6.92 (95% C.I. 6.68 – 7.16) per household per month in the intervention and control groups, respectively and there was no significant difference between the treatment arms: Odds Ratio 1.68 (95% C.I. 0.32 – 84.25, $P = 0.803$) and this remained constant for the duration of the study period.

Table 4:2 Assessment follow-up of households, repellents use and perceptions during the study period

	Repellent n (%)	Placebo n (%)	Total proportions/treatment	P value
<i>Like repellent</i>				
Yes	462 (99.35%)	390 (84.41%)	852 (91.91%)	<0.0001
No	3 (0.65%)	72 (15.59%)	75 (8.09%)	
<i>Compliant</i>				
Yes	379 (81.50%)	361 (78.13%)	740 (79.83%)	0.202
No	86 (18.49%)	101 (21.86%)	187 (20.17%)	
<i>Perceived effectiveness</i>				
Yes	457 (100.00%)	379 (84.03%)	836 (92.07%)	<0.0001
No	0 (0.00%)	72 (15.96%)	72 (7.93%)	

4.6.3 Post-study survey

The main reason for non-compliance to interventions was forgetfulness, with 70% (n = 35) of the households in the intervention and 60% (n = 89), (p = 0.241) in the control arm reporting that the major reason they did not comply with the intervention at some point during the study was because they forgot to apply the repellent. Travel also led to non-compliance with 26% of households in the intervention arm and 37.83% of households in the control arm not complying for a month because they had gone to work in the fields.

When asked why they liked using the repellents, significantly more households in the intervention arm 98.69% (n = 455) relative to the control arm 45.56% (n = 208) cited effectiveness, (p = <0.0001). It is worth noting that all households who mentioned nice smell and smooth feeling on the skin as reasons for using repellents were from the placebo arm of the trial. When asked if anyone in their household suffered from malaria during the trial, significantly more participants from the placebo arm answered yes: 32.9% versus 15.5%, (p < 0.0001).

Equal proportions of households were willing to continue using repellents after the clinical trial (Table 4:3). When asked if they would be willing to pay if the repellent was made available at a fee, 99.78% (n = 458) of the households in the intervention and 98.48% (n = 455), (p = 0.999), in the control arm reported that they were ready to pay a small fee, with majority of the households in the intervention, 84.34% (n = 388) and control arms 87.77% (n = 402), (p = 0.347) willing to pay at most \$ 0.30 for a tube of repellent (Table 4:3), even though all participants perceived that the value of the repellent was at least double that figure. There was no difference in willingness to pay when SES quintiles were compared (p = 0.668).

Table 4:3 Assessment of perceptions on repellent use, effectiveness and cost after the study period

	Repellent n (%)	Placebo n (%)	Total proportions/treatment	P- value
<i>Reasons for non-compliance</i>				
Forgot	35 (70.00%)	89 (60.13%)	124 (62.63%)	0.241
Away in the field	13 (26.00%)	56 (37.83%)	69 (34.85%)	
Don't like repellent	1 (2.00%)	-	1 (0.51%)	
No mosquitoes	1 (2.00%)	2 (1.35%)	3 (1.52%)	
Ran out of repellent	-	-	-	
Other	-	1 (0.67%)	1 (0.51%)	
<i>Perceptions about repellents</i>				
Effective	455 (98.69%)	208 (45.61%)	663 (72.30%)	<0.0001
Easily available	5 (1.08%)	50 (10.96%)	55 (6.00%)	
Nice smell	-	99 (21.71%)	99 (10.80%)	
Smooth on skin	-	98 (21.49%)	98 (10.69%)	
Other	1 (0.21%)	1 (0.21%)	2 (0.22%)	
<i>Willingness to use repellent again</i>				
Yes	462 (99.78%)	463 (99.78%)	925 (99.78%)	0.999
No	1 (0.21%)	1 (0.21%)	2 (0.22%)	
<i>Willingness to pay</i>				
Yes	458 (99.78%)	455 (98.48%)	913 (99.13%)	0.034
No	1 (0.21%)	7 (1.51%)	8 (0.87%)	
<i>Perceived cost of repellent</i>				
< 0.6 USD	99 (21.80%)	111(26.74%)	210 (24.17%)	0.023
0.6 – 1.2 USD	280 (61.67%)	212 (51.08%)	492 (56.62%)	
1.2 – 1.8 USD	61 (13.43%)	75 (18.07%)	136 (15.65%)	
1.8 – 3.05 USD	13 (2.86%)	17 (4.09%)	30 (3.45%)	
> 3.05 USD	1(0.22%)	-	1 (0.12%)	
<i>Amount participants were willing to pay</i>				
< 0.30 USD	388 (83.43%)	402 (87.77%)	790 (86.06%)	0.347
0.30 – 0.60 USD	64 (13.91%)	52 (11.35%)	116 (12.64%)	
0.60 – 1.20 USD	7 (1.52%)	4 (0.87%)	11 (1.20%)	
1.20 – 1.52 USD	1 (0.21%)	-	1 (0.11%)	

4.6.4 Focus group discussions

4.6.4.1 Perceptions around malaria control and transmission

To provide a general picture of the community's knowledge, attitude and practice in relation to malaria and ways to control malaria, participants were questioned about their knowledge of malaria transmission and methods of prevention and control used. Some of the participants had a comprehensive understanding of malaria and control, as observed from the response of one female respondent below: "*Malaria is caused by a female mosquito when it bites you at midnight*" (Meeting group 5, 16th June 2013).

Interestingly however, and especially in a region where there has been consistent malaria control, research and intervention implementation by both non-governmental and governmental organizations for over 20 years [15-17], the community members did not appear to have an in depth knowledge of malaria transmission. In trying to assess the depth of community knowledge on the malaria transmission process, the respondents were asked how many times a mosquito had to bite a person for it to transmit malaria. Most of the respondents did not seem to know:

"*We do not know unless you tell us*"- (Meeting group 4, 14th June 2013).

"*Many times*"- (Meeting group 1, 14th June 2013).

This indicates the community knowledge on malaria transmission is superficial, so that whilst the community is aware that mosquitoes transmit malaria, their knowledge on this transmission process is scant. These gaps in knowledge might suggest a bias during implementation of malaria control programmes, so that, rather than promoting community sensitization and education on the objectives of the intervention, the link between intervention and disease, and the benefits of the intervention to the individual

and the community, these programmes likely focus more on coverage of the control tools.

4.6.4.2 Preference of malaria prevention tools used in the community

All respondents had used some form of personal protection against mosquito bites even for those who weren't quite sure what malaria was. It also emerged that they had been using these tools for a long time and were convinced that the tool each one of them had been using was the most effective. The most commonly reported malaria prevention tool used was the bed net, when respondents were asked which tool they used to protect themselves from mosquitoes and malaria:

“We use nets” – (Meeting group 1, 14th June 2013).

Even though some of the respondents were aware of mosquito repellents and/or had acquired topical and spatial repellents at some point in the past 2 years, during or after the clinical trial, most of them still preferred using the bed net;

“I would prefer the net” – (Meeting group 3; 25th June 2013)

When the respondents were questioned on why they preferred the bed net to other mosquito control tools, two major reasons were given. The first was cost effectiveness:

“Because mosquitoes will not bite you when you are sleeping under a net but for the repellents they last for a short time and when the smell wears off then the mosquitoes bite you” – (Meeting group 2, 26th June 2013).

The second was generally the ease of use:

“MG: FR: 03: Because it is not cumbersome”- (Meeting group 3, 25th June 2013).

4.6.4.3 Familiarity of topical repellents

At the onset of the FGDs, most respondents’ awareness of repellents was thin, with almost half of them largely unaware of topical repellents as a malaria control tool. However, those who had heard of topical repellents had adequate knowledge on the proper technique of using/applying the repellents as illustrated by the following quotes when respondents were asked how repellents were used;

“You can apply and then it stays for a few hours after that it is no longer effective and the mosquitoes can bite you. After you apply it you have to wash your hands well with soap” – (Meeting group 5, 16th June 2013).

For those who knew about repellents, the primary source of information was outreach from the Ifakara Health Institute (IHI), previously Swiss Tropical Institute Field Laboratory (STIFL), which is the institute under which the clinical trial project was conducted. When asked how they came to know of repellents most respondents mentioned the clinical trial, which distributed the repellents free of charge:

“They were being distributed by people from STIFL (IHI)” – (Meeting group 1, 14th June 2013).

4.6.4.4 Reported experience of use after topical repellent distribution and use

After repellent distribution, all respondents reported that they had used the repellent intervention issued to them during the second phase of FGDs. The most commonly reported reasons for continued use of the repellents by the respondents were mainly

because of their effectiveness against mosquito bites and also because of the appeal in odour and presentation:

“ I liked it because it prevented mosquitoes and its smell did not affect us in any way like causing flu or any other effects”- (Meeting group 3, 20th June 2013).

Another reason that emerged from the interviews was that every member of the household could use the repellent as opposed to other interventions issued which only a few household members used:

“ I would choose the applying repellent because it can be used by the children, my husband and even visitors”- (Meeting group 3, 20th June 2013).

There was one report of side effects to repellent use, however this was during the clinical trial and not in the FGD study:

“Yes I know my sibling he used to get rashes all over the body so he was told not to apply the repellents anymore”- (Meeting group 5, 16th June 2013).

4.6.4.5 Preference for different applications of repellents

After exposing the respondents to typical topical repellents containing active ingredients such as DEET and PMD and in various formats such as lotion, jelly, spray, permethrin impregnated clothing, (*kanga*), transfluthrin impregnated sack cloth and permethrin impregnated net fencing, the respondents expressed the following views and preferences;

“I found the smell to be too strong” when asked about DEET in spray format – (Meeting group 2, 25th June 2013).

“I liked the smell “ when asked about PMD in lotion and spray formats – (Meeting group 2, 25th June 2013).

“I did not like the smell because it was too strong “ when asked about DEET in jelly format – (Meeting group 2, 25th June 2013).

“The applying repellent because everyone can use it but the kangas cannot be used by everyone” when asked to choose between topical repellents and insecticide treated clothing ‘kanga’ – (Meeting group 3, 20th June 2013).

“If you sat near the sack repellent then the mosquitoes couldn’t bite you but if you sat just a distance away then they would bite”– when asked about the transfluthrin impregnated sack, (Meeting group 6, 20th June 2013).

“I got the net so I used to sit inside it and the mosquitoes were very few. They used to bite the feet only but I could stay for like half an hour without bothering with any mosquitoes” – when asked about the permethrin impregnated net fencing, (Meeting group 6, 20th June 2013).

4.6.4.6 Factors that determine the continued use of topical repellents

For those who did not use repellents during the clinical trial, repellents were generally not popular. There were a several barriers to repellent use in this community; the first being access to the repellents:

“We were given repellents for applying but after they got finished I have not used anything else apart from nets” – (Meeting group 1, 16th June 2013).

Repellents were provided for free during the clinical trial. However after the clinical trial, the community was unable to access repellents as they were not available in shops and drugs stores in the study area, as was highlighted by the respondent above.

The cost of the repellents according to most respondents limited their affordability with most respondents prioritizing other living essentials over the repellents. When asked to choose between buying a soda or the repellent (subject to availability), most of the respondents opted to buy the refreshment:

“I would buy the refreshment or a net otherwise I would just use a lot of clothing to cover myself” – (Meeting group 1, 16th June 2013).

4.6.4.7 Community recommendations on improving repellent use

In an effort to understand how to improve the use of repellents, participants were questioned on what they felt was necessary to make the interventions better. While most of the responses revealed that the repellent application was fine the way it was, other recommendations included the cost of the repellent:

“I wouldn’t buy them because that is expensive unless you sold them in 500 shillings bottles” – (Meeting group 1, 16th June 2013).

It should be noted that the bottle the respondent was recommending to be sold for 500 TZS/\$0.30 contained 120 ml of repellent.

Odour of DEET repellent:

“I did not like the smell because it was too strong” – (Meeting group 2: Male respondent).

Issuing extra insecticides so that they could re-treat the impregnated clothing issued:

“I also think that you should give us repellents for the kangas so that we can treat them once we wash them” – (Meeting group 3, 25th June 2013).

Table 4:4 Major themes generated from the focus group discussions (FGD's) and small group interview (SGI)

Major results theme	
Theme 1	Respondents were aware of the link between malaria and mosquitoes, but their knowledge on malaria aetiology and transmission was shallow. This did not however, effect their compliance with an intervention that was available free of charge
Theme 2	Although respondents had adequate knowledge of repellents as a mosquito control tool, they preferred to use the bed net over repellents
Theme 3	Those respondents aware of topical repellents had adequate knowledge on their proper use
Theme 4	Availability (access) and cost of repellents were major barriers to repellent use after the trial ended and repellents were no longer supplied
Theme 5	The respondents perceived the repellents to be effective against mosquito bites, mostly in the early evenings
Theme 6	Respondents recommended repellents be made more available and insecticides (permethrin) used to treat clothing be provided to enable self treatment

4.8 Discussion

Despite the proven efficacy and acceptability of repellents for prevention of malaria [7-10], knowledge and utilization of repellents as a malaria control tool is low in sub-Saharan Africa. Lack of awareness of repellents as a malaria control tool is one of the major barriers to repellent use in sub-Saharan Africa. As observed from the baseline survey at the start of this study, most respondents had not used repellents before the implementation of the clinical trial. Therefore, use of topical repellents was completely new in this community similar to several other studies conducted in the African continent [24-26]. It is evident that improving community knowledge and

awareness [25-28] as well as retooling interventions to community needs and preferences [29] will improve the acceptability and uptake of interventions being advocated. The most commonly used malaria control tool in the study area was bed net. Social marketing of LLINs started in Kilombero and Ulanga district in July 1997, under the KINET project. At the launch of this programme the community was educated on malaria transmission and control [15]. This campaign was followed by the launch of the Tanzania National Voucher System (TNVS), implemented by the National Insecticide Treated Nets programme (NATNET), under the National Malaria Control Programme (NMCP) of Tanzania, from 2004. In 2007, the Ministry of Health and Social Welfare (MoHSW), collaborating with other partners launched the under five Catch-up Campaign, parallel to the TNVS programme. In 2008, the MoHSW and partners launched the Universal Coverage Campaign (UCC) [30]. Therefore, if repellents and indeed any other novel tools are to be accepted and used to complement LLINs and IRS, there will be a need for social marketing, community education and sensitization to be employed for a substantial period of time. It is also essential to determine community preferences. Tools that require daily compliance are initially likely to have limited uptake, as the community has to remember to adhere to them on a daily basis. As observed from the FGDs study, ease of use was one of the reasons why the community preferred bed nets to repellents. This was because, once hung, the bed net was used over a long period of time as you simply pull it down when you get into bed, compared to having to remember to apply the repellent every evening. However, ease of use was not the only factor that effected compliance. In the follow-up surveys, it was observed that there was lower compliance in the control arm relative to the intervention arm. Likewise, in the after study, it was observed that more households in the intervention arm relative to the control arm used the repellent

because it was effective. This finding demonstrates that compliance to interventions does not only depend on its availability and ease of use but also on its effectiveness.

In the FGDs it was also observed that even though sisal impregnated sisal strips did not require daily compliance and were easy to use, they were reported to be effective over very short distances, and this discouraged the community from using it.

These finding demonstrates that to impact compliance, the efficacy of the tools being recommended need to be established. A recent mathematical model demonstrated that the effectiveness of any repellent is extremely dependent on two factors: efficacy and compliance (Moore and Briet, in preparation). The most effective tools are those that have high efficacy and require little user compliance such as house screening [31].

The major reason for use of topical repellents by the community in Mbingu village is to prevent nuisance biting by mosquitoes. Although a proportion of the community could associate mosquito bites with malaria, the results of this study imply that they used repellents to avoid being bitten by mosquitoes rather than to avoid contracting malaria. These results were similar to a study carried out in a coastal community in Mexico, where 80% of the respondents said they allowed IRS in their households to reduce mosquito bites while only 2% said they allowed IRS to avoid contracting malaria [32]; and in rural Tanzania, where respondents reported that main reason for using LLINs was to prevent mosquito bites: 73% of the respondents reported they allowed IRS in their households to reduce mosquito bites and only 17% related protection from mosquito bites with reduction of malaria in the family [24]. These findings demonstrate that tools being advocated as interventions, especially in malaria control should address both short and long-term goals, i.e. address the problem of nuisance biting or mosquito densities (efficacious to enhance uptake) as well as

reduce disease prevalence/incidence in the long run (resultant effectiveness). This is likely to encourage uptake and acceptability as opposed to tools whose benefits are realized in the long-term, and highlights the need to test new vector control interventions against nuisance biting insects as well as target vectors during development for a better understanding of how effective that tool will be in the real world for disease control purposes.

The major reason for not using repellents in this community was reported to be lack of knowledge of repellent use and is similar to findings in other studies [25], where low repellents use was associated with poor knowledge of repellents. Availability of repellents in this community was another barrier to repellents use as observed from the baseline survey.

Also, in the FGDs, after the clinical trial, when asked why they did not use repellents, the respondents cited availability as a barrier, reporting that they did not know where to access repellents. Observations carried out by the study investigators during the clinical trial and FGDs, indicated that no topical repellents were available in the shops and drug stores in the study area. Therefore, despite most households indicating willingness to continue repellent use, and even pay a small fee, access to repellent was a major barrier to repellent use.

Another barrier to the use repellents was cost [33]. During the FGDs, even though all respondents were aware of repellents as a mosquito control tool, they all preferred using LLINs as they reported that repellents were more expensive in the long run because they had to be replaced every end of the month compared to LLINs, which could last up to five years before replacement, if well taken care of. This finding was consistent with outcomes from other KAP studies assessing uptake of interventions

[24,27,34]. As seen from the above studies, cost of mosquito control interventions greatly influences the acceptability and uptake in communities where they are to be employed. In rural and urban areas in Tanzania, a 150 ml bottle of 15% DEET repellent costs USD \$1.00. On average, respondents were willing to pay \$0.32 for a 150 ml tube of repellent that would last one adult less than one month. The current price of repellents is too expensive for the subsistence farmer, who lives on \$1.50 USD per day. Therefore, even though incorporation of repellents into malaria control programmes on a community scale, is likely to use a cheaper but efficacious option of repellent, as was the case in Ghana [10], it is unlikely that the repellents would be subsidized down to or lower than \$ 0.32. Also, scale up and extensive use of repellents under programmatic conditions as well as emergence of a repellent market is bound to drive the cost of repellents down. However these cost are unlikely to be lower than the cost of delivering a single LLIN, which costs USD \$5.30 and protects two people for up to 5 years (\$0.50 per person per year) [30]. Therefore if we are to encourage up take of repellents as a malaria control tool, the cost needs to be greatly reduced, potentially through government and non-governmental organizations offering subsidies on repellents following the example of LLINs [23]. The government may also encourage local production of repellents through tax exemption for local repellent manufacturers.

From the FGDs, it was observed that knowledge on malaria transmission and control was relatively superficial. While most respondents associated mosquitoes with malaria, when probed, few were able to detail processes of transmission, aetiology and prevention in any depth. Therefore, although all respondents from the FGDs reported that they used the repellents issued, it is likely that they did so with only a superficial understanding of the objectives of using repellents. This might have been

because the community was more concerned with preventing mosquito bites than contracting malaria, as observed in other studies [24,32]. As all respondents reported that they had complied with repellent issued it was not possible to assess the relationship between compliance and level of knowledge of malaria transmission. The superficial knowledge of malaria transmission observed in this community underscores the importance of incorporating community education and sensitization before implementation of any intervention to achieve its desired objective. Social marketing the product, and neglecting key messages regarding how these interventions benefit the communities in which they are being implemented, is likely to negatively effect uptake of that intervention. It is therefore essential for the community to be involved in designing and implementation of intervention programmes so that they have a better understanding of the objectives and use of tools being employed.

Several studies have shown that there has been better uptake of interventions in communities where awareness and sensitization have been conducted [35]. Promoting knowledge and awareness also deters any misconceptions that the community may have towards a particular intervention and it is essential for effective implementation of that intervention [36,37]. During FGDs for this study, some respondents reported that they had 'heard' that LLINs caused infertility and also claimed that if/when they use repellents then their skin pores will be blocked and they will get sick. However in a KAP study in Rukungiri, Uganda, women who had previous knowledge of the use of ivermectin were more involved in making decisions of how ivermectin should be distributed to the community compared to those women who had no prior knowledge of this drug [38]. It is therefore essential to acknowledge and address the community's misconceptions and misinformation about intended interventions in

order to improve acceptability, uptake and effectiveness. Rather than the implementing organizations solely marketing the product to achieve extensive coverage, it is beneficial to also educate the community on the safe use of these interventions and the correlations between their products, the disease and its benefits.

The respondents' preference of LLINs to repellents is attributable to cost effectiveness, convenience of use and availability. The major reason given for non-compliance to repellent use was that the respondents 'forgot' to use it, while ease of use was ranked second among reasons why respondents preferred using bed nets. It was cumbersome to remember to re-apply the repellent after every few hours, unlike simply sleeping under a LLIN. Repellents should therefore be presented in a format that will encourage uptake. As the major occupation in the study area is subsistence farming, most community members bathe in the evening after coming from their farms. Repellents can be incorporated into body lotions so that they are applied after taking the evening bathe. Repellents can also be impregnated in clothing, especially in *kangas* used by women in the evening when cooking outdoors. Development of tools that do not require daily compliance such as long lasting spatial repellents that act over long distances should also be explored [39].

Respondents also preferred LLINs because it protected them when they were asleep and vulnerable to mosquito bites as opposed to when they were awake and could chase mosquitoes away. The community however preferred to use repellents in the early evenings when sitting outside their houses to have a chat with other family members and friends without being bothered by mosquito bites. This finding is important because it suggests that repellents can be used complimentary to LLINs in

the early evening, before LLINs are employed, which was the major objective of this study.

Perceived irritating odour of DEET topical repellents reduced its use by the community in this study, a finding similar to studies in North Tanzania and Mexico where participants refused to use IRS because of the 'bad smell' of insecticides used [24,32], emphasizing that interventions should be tailored to be perceived as pleasing by users. PMD was perceived as pleasant as found in several other studies [7,10,40].

The most salient recommendation that came out of this study was that interventions advocated to the community should fit the community needs, such as providing repellents that have a pleasant smell and feels good when applied to the skin. Respondents that were issued with Permethrin impregnated *kangas*, reported that even though effective, it only protected a single individual at a time and suggested that all members in the household be issued with a treated *kanga*, and like LLINs, be issued with the 'chemicals' (insecticides) used for re-treatments so that when the effect of the insecticide was diminished they could treat the clothing on their own. Insecticide Treated Clothing (ITC) has been successfully implemented in other settings [41-44] and therefore this tool would easily be introduced in this community. Another outcome of this study was the effectiveness of the topical repellents that were issued. The respondents found topical repellents to be effective in protecting against early evening biting outdoors. This finding is similar to other studies, where repellents have been used to protect against vectors biting outdoors and by extension reduce the incidence of malaria [7,8,45]. Therefore both topical repellents and ITC, if designed to meet the needs and preferences of the community, could offer potential

interventions that could be introduced for malaria control and would be readily accepted by the community.

4.9 Conclusion

In this setting, the major limitations to use of repellents, similar to those identified from other studies were lack of knowledge, availability of repellent, cost and need to remember to use it every evening or even more than once in a single evening. While the community was highly knowledgeable about malaria, their knowledge was found to be superficial, indicating poor community education and sensitization. Although currently LLINs are the most commonly used and preferred malaria prevention and control tool, their introduction to the community was initially marked by similar limitations emerging from this study such as the need to use it daily and the cost being prohibitive. When repellents were provided free of charge to all trial participants compliance was high. It is therefore likely that uptake will improve if accessibility of repellents is improved through lower costs and greater availability through the commercial sector, comprehensive social marketing and community sensitization on use of repellents, as well as delivery of repellents in formats that respond to community desires. Even though LLINs were the preferred mosquito protection tool, the community saw a benefit in the use of topical repellents in the early evening, especially to prevent mosquito nuisance indicating the potential of using repellents complimentary to LLINs. However, longer lasting repellents are an essential requirement to avoid the need for frequent reapplication that most people find off-putting. The difference in compliance reported during and after the study is likely due to recall bias at the end of the study. Other avenues such as long lasting spatial

repellents might be used if they are effective enough to protect the peridomestic space occupied by the family and visitors in the evening.

4.10 Limitations to the study

A ranking of repellent preference had previously been reported in this study, but as there were too few repellents types/formats to issue to each FGD participant, these results were discarded along with some themes that had earlier been reported as they did not represent true results of community preference to different repellent formats.

As the participants were only issued with one repellent, it was not possible to explore whether the participants would use the repellents complimentary to each other if they had been issued with different formats of repellents. However, findings from the FGDs indicated the community members used the tools complementarily.

Another limitation of this study is that compliance, during the follow up and post-study surveys, was established by self-reporting of use by the study participants. It was not logistically possible to observe compliance of households to repellents use for each household every evening and therefore observed and reported compliance could not be compared, and this should be taken into consideration when interpreting the results of this study.

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Chapter 5: Literature review: Evaluation of repellent efficacy in reducing disease incidence

5.1 Background.

Repellents, used throughout pre-history [1] are currently used by millions of people worldwide to prevent nuisance bites from blood-feeding insects, and is now a multi-million dollar, global industry [2]. Several groups of animals including passerine birds and white faced capuchin monkeys anoint themselves with leaves, fruit and even millipedes that contain compounds that are proven deterrents of ticks and mosquitoes [3, 4]. This behavior is observed to increase at times when attack from such arthropods is higher as observed in capuchin monkeys of South and Central America [5]. This observation is an indication that the use of personal protection from blood-feeding arthropods must improve the biological fitness of the animal that applies such repellents through reducing energy expended on “host defensiveness” or reducing its susceptibility to arthropod borne diseases [6]. However, until recently, there has been limited scientific evidence on the efficacy of repellents to reduce disease.

The majority of research into the highly effective mosquito repellents available today was carried out by scientists employed or funded by the military to protect troops stationed in high disease risk areas. Some of the world’s most important programs into the understanding and prevention of insect borne disease have risen as a result of conflict in tropical regions that lead to massive loss of life from diseases such as yellow fever, louse borne typhus and malaria [7]. Two of these discoveries: DEET, a topical repellent [8] and long lasting permethrin treated clothing [9] will be reviewed in this chapter. Two other repellents will also be reviewed: Para-methane 3,8 diol (PMD), a topical repellent discovered in China [10] and mosquito coils that were

developed by the private sector in Japan [11] and are an example of area or spatial repellents.

Topical repellents are oils or lotions applied to the exposed skin or clothes of the consumer, with the most safe and effective being DEET, Picaridin and PMD.

Picaridin will not be reviewed here, because there is, to date, no epidemiological evidence of its efficacy, although a well-designed trial to evaluate its efficacy against malaria is currently underway with results available in 2014 [12]. Permethrin treated clothing is clothing impregnated with a safe pyrethroid insecticide and binding agent to allow the permethrin to adhere to the fabric even after several washes. Permethrin is a synthetic pyrethroid, which has been extensively tested by the Military [13-16] and is the only insecticide approved for this use category by the United States Environmental Protection Agency (USEPA) [17]. It is non-staining, odorless, resistant to UV-light and safe for regular use and is therefore an excellent tool for longer-term prevention of arthropod bites. Mosquito coils are spiral shaped products made from organic fillers, binders and additives that allow the organic components to smolder evenly and continuously, to which a volatile pyrethroid insecticide is added that evaporates as the coil smolders over several hours after it is ignited. They are classified as area (spatial) repellents. Spatial repellency is used here as a general term to refer to a range of insect behaviors induced by airborne chemicals that result in a reduction in human-vector contact. This can include knock-down, interference with host detection (attraction-inhibition) or movement away from a chemical stimulus [18]. Other forms of spatial repellents include vaporizers and mats that have available extensive phase II (laboratory) data demonstrating excellent efficacy [19] but no epidemiological evidence of efficacy to date [20]. Vaporizers and mats require electricity to evaporate insecticide from a small liquid reservoir containing insecticide

or cellulose mat impregnated with insecticide, respectively. This feature limits their application for disease prevention in the rural tropics where the majority of vector borne disease occurs, since electricity is not available. Another intervention of note is passive emanators that have a large surface area allowing the passive diffusion of insecticide from the surface. There is extensive evidence from studies with dichlorvos that passive emanation of insecticides is effective against malaria vectors (Table 5:1). However, dichlorvos does not have a suitable toxicity profile for use for public health [21]. The discovery of the extremely non toxic pyrethroid insecticides, metofluthrin and transfluthrin, means that passive emanation of such compounds is an area of current research interest [22, 23] and large scale epidemiological trials will begin in the near future. Development of such products will be of great value because while the pyrethroid insecticides used in the coils is not known to be harmful to humans, often the smoke produced from combustion of coils is a nuisance to people reducing consumer acceptance and some brands generate Products of Incomplete Combustion, (PIC), which are harmful to humans [24, 25].

The annual market value of personal protection consumer products is over \$2billion for powders, gels and repellents and \$2.6 billion for spatial repellents including vaporizers and coils. It is estimated that 45 to 50 billion mosquito coils are used annually by approximately 2 billion people worldwide [26], mainly in Southeast Asia, but with a growing market in South America and Africa. These products present a great opportunity for public health, because such products could provide a means of disease control that is already proven highly acceptable to end-users, since those that can afford them are willing to buy them.

5.2 Vector behaviour modification for disease prevention

The World Health Organization (WHO) has recommended that all travelers to disease endemic areas should minimize exposure to insect bites by selecting a combination of personal protection methods including insect repellents, mosquito nets, mosquito coils, aerosol sprays, protective clothing, screening and air-conditioning [27]. The United States Department of Defense (DoD) spent \$4 million in developing the insect repellent system that comprises the proper wearing of a permethrin treated uniform, and the application of extended-duration DEET lotion to exposed skin which, provided it is used correctly, provides close to complete protection from arthropod-borne diseases [28]. However, there has been no discussion on the implementation of repellents for public health use. The main explanation behind this is that until recently, there were insufficient studies conducted to convincingly demonstrate that repellents can be effective against disease transmission.

Public health vector control tools such as indoor residual spraying (IRS) and the use of Long lasting insecticide treated nets (LLINs) are extremely effective in sub-Saharan Africa [29]. Massive mobilization of both financial and political resources of the past decade [30] has resulted in scale up of LLINs and IRS and has had a great impact on malaria transmission [31]. However, there is a substantial amount of disease transmission both within and outside of Africa, where vector behavior evades control through conventional means such as insecticide treated materials, because vectors bite outdoors and at times when people are still active (Table 5:2 and 5:3). Recent estimates are that 16% of global malaria burden and 8% of malaria mortality occurs outside of Africa [32], while outbreaks of dengue and other arboviruses are increasing and spreading geographically [33]. Thus, tools targeting these outdoor and day biters are required. With the new impetus for malaria eradication of the past

decade and the realization that the existing control tools LLINs and IRS cannot solely achieve this, repellents are increasingly being considered as the supplementary tool in appropriate scenarios [34]. Modern repellents are extremely effective at preventing man-vector contact. The burden of vector-borne disease remains elevated despite substantial gains in control. There remains a challenge to develop repellency as a vector control option to complement existing tools in scenarios where the vector [35] (Table 5:2) or the human population [36] (Table 5:3) exhibit behaviors that require their use.

5.2.1 How repellents work to reduce vectorial capacity and vector borne disease

When considering vector control for disease prevention it is useful to consider how repellents could reduce the vectorial capacity of the disease vector population of interest and thus reduce disease transmission. The concept of vectorial capacity was derived from models of malaria transmission first devised by Ross, and was developed to guide the first global malaria eradication plan [37]. Vectorial capacity is described by an equation (Figure 5:1) and is defined as “the average number of inoculations with a specified parasite, originating from one case of malaria in unit time, that the population would distribute to man if all the vector females biting the case became infected” [38].

$$C = \frac{ma^2bp^n}{-\ln p}$$

C = new infections disseminated per person per day by each mosquito
m = number of mosquitoes per person
a = probability a vector feeds on a host /day i.e the proportion of females feeding on man divided by the duration of the gonotrophic cycle in days
ma = the number of bites/man/day
p = probability of daily vector survival
1/-ln^p = duration of the vector's life in days once it has survived the intrinsic incubation period
n = duration of the extrinsic incubation period in days
b = proportion of sporozoite positive mosquitoes that are infectious

Figure 5:1 Equation describing vectorial capacity

The concept of vectorial capacity is sufficiently simple that it can be applied with some modifications to account for varying vector behavior, competence, and ecology as well as differences in the dynamics of infection, disease, and immunity in vertebrate hosts, and has been used to understand transmission of other vector borne diseases including dengue [39] bluetongue [40] onchocerciasis [41, 42] bancroftian filariasis [43, 44] and schistosomiasis [45]. Vectorial capacity describes the potential intensity of transmission by mosquitoes as a function of 1) the man biting rate, representing the incidence of biting contact between the mosquito and humans in terms of the number of bites per person per day and indicates the number of vector females that could become infected per case per day 2) the expectation of infected life, which is days of infective life per mosquito infected with the given parasite species and 3) the man biting habit, which is bites on a person per day by an individual female mosquito [46] all of which can be simply measured using standard field collection techniques [47]. This exceedingly elegant means of considering the

process and impact of vector control on man vector contact and mosquito survival has been verified with field data [38] and provides a convenient logical framework to consider the impact of new vector controls. The majority of work involving the vectorial capacity equation has considered insecticides that reduce both mosquito numbers and life expectancy of mosquitoes and have an excellent impact on reducing malaria intensity. However, in the original paper where VC was described the author demonstrated that by reducing man-biting rate by 50% there was a consequent 75% reduction in the vectorial capacity of the mosquito population [46]. Vectorial capacity is extremely sensitive to changes in the biting rate because a vector needs to bite twice in order to obtain and then transmit a pathogen – hence man biting is squared in the equation (Ma^2). Thus the use of repellents will have a strong effect on overall vectorial capacity by reducing the probability of infecting or being infected by a vector – as described by Ma^2 . Therefore, when considering disease control we will define repellents as those interventions that reduce man vector contact without killing a large proportion of the vector population, i.e. those interventions that keep the human population and the vector population apart.

5.3 Randomised Controlled Trials for measuring the disease impact of repellents

Different kinds of evaluations have been conducted to determine the effect of repellents on disease incidence. Randomized controlled trials (RCTs) are currently considered to be the gold standard for testing the effectiveness of interventions for disease reduction in a population [48], provided that they are well conducted [49]. The most important feature of an RCT is that those individuals recruited into the trial are randomly assigned to the intervention or a control thereby minimizing selection and allocation bias to control as much as possible for both known and unknown

confounders that could influence the correct measurement of impact of the intervention [50]. Other advantages of well-conducted RCTs are that it facilitates blinding of treatments, from investigators, participants and assessors to prevent bias in estimation of intervention effect [51]. It allows for the use of probability theory that any difference seen between the different arms outside the treatment effect is due to chance. There is a large body of guidance available to researchers on the importance of correct trial design [52], implementation [53-55] and reporting [56].

The main disadvantage of RCTs is the limitation of external validity i.e. the results of the RCT may not be applicable to the general population, due to differences in geographical location, characteristics of patients recruited, trial procedures and methods of measuring the outcomes in the trial. For this reason, it is advised that standard methods to ensure quality and reporting guidelines are followed that will allow systematic review and meta-analysis which aims to collate and synthesize data from multiple studies that meet pre-specified eligibility criteria using methods that attempt to minimize bias [52]. The other disadvantages are cost and time. RCTs are quite expensive [57] and takes several years until the results are published; thus, they may be less relevant at the time of publication [58]. However, when considering the public health implementation of a new vector control product, the investment in RCT is small when considering the importance of implementing a proven intervention that will save lives rather than wasting money on implementing an ineffective intervention (Christian Lengeler, pers. comm.). The cost of the series of RCT used to generate evidence that bed nets prevented malaria [59] was less than \$10 million; but between 2004 and 2010, \$17 billion was spent on bed nets [60].

5.4 Randomized controlled trials of topical repellents

5.4.1 Southeast Asia

In a refugee settlement in Pakistan, a household randomized trial of Mosbar, (a soap containing 20% DEET and 0.5% permethrin that is lathered on but not rinsed off) vs. a placebo lotion demonstrated a 56% reduction in *Plasmodium falciparum* (*P. falciparum*) malaria, Odds Ratio (O.R)=0.44 (95% C.I. 0.25 – 0.76, P = 0.004) and a non-significant effect on *Plasmodium vivax* (*P. vivax*) malaria O.R.=1.29 (95% C.I. 0.86 – 1.94, P = 0.226) [61]. The study was carried out on a waterlogged land endemic for malaria and transmission was effected by *Anopheles culicifacies*, *Anopheles stephensi*, *Anopheles nigerrimus* and *Anopheles pulcherrimus*, which are predominantly early evening biting vectors [61]. This characteristic makes topical repellent use ideal as it is applied in the early evening coinciding with peak activity of these vectors. This local vector bionomic may have meant that the repellents reduced a substantial amount of malaria transmission and demonstrated the importance of studying the local vector bionomics to determine if the proposed intervention will have any impact on the vector population. The study employed simple randomization to allocate treatment to the participants. Randomization minimizes the allocation bias of the treatments and confounding factors that have not been taken into account. Passive case detection of malaria cases was used, which might have led to loss of cases that did not report to the health clinic. Compliance was established by self-reporting of use, every fortnight and therefore could not be conclusively ascertained. Field staff, laboratory technicians and participants were blinded to the intervention. Even though this study demonstrated an effect of repellents, it did not take into account the whole malaria transmission season. This study took place for only 6

months, during *P. falciparum* transmission season and therefore demonstrated an effect only against *P. falciparum* malaria. No effect was shown against *P. vivax* malaria because study was carried out when transmission of *P. vivax* malaria was low and there weren't enough cases to demonstrate a treatment effect. This study would have been stronger if it had been carried out for longer to take into account both *P. falciparum* and *P. vivax* malaria transmission seasons. As *P. vivax* malaria is known to recrudescence, the study investigators should have cleared all malaria cases through an appropriate treatment regimen after checking for glucose-6-phosphate dehydrogenase (G6PD) deficient individuals [62], so that any cases that were observed would be classified as new malaria cases and not recurrent *P. vivax* cases. Thus, the investigators would have avoided losing malaria cases that they classified as recrudescence cases while they were actually new cases, which reduced the power of the study. It would also have been prudent if the investigators had employed active case detection, where they visited all households recruited into the study and screened for malaria, instead of waiting for study participants to report to the camp's health facility. This way, the investigators would have captured malaria cases of those individuals who visited alternative health facilities or chose to buy drugs directly from the drug stores. Active case detection would also allow for inclusion of individuals who were too weak to visit the health facility for treatment or found the facility to be too far to seek services. Compliance could also have been better established by conducting frequent spot checks to determine if the study participants did indeed use the treatments they were issued.

In another refugee camp in Thailand, a double blind, randomized clinical trial on the effect of DEET mixed with *thanaka* (a root paste made from pulp of the wood apple tree, *Limonia acidissima*, used locally as a cosmetic) compared to *thanaka* alone in

pregnant women demonstrated a 28% reduction in malaria incidence: 10.6% (95% C.I. 7.5% - 13.5%) in women who used *thanaka* + DEET, compared to the arm that used *thanaka* alone 14.8% (95% C.I. 9.9% - 19.7%) in *P. falciparum* malaria although the difference was not statistically significant [63]. There was also no significant difference in the transmission of *P. vivax* malaria between the 2 treatment arms. The lack of a treatment effect was most likely due to malaria transmission being too low to demonstrate a treatment effect as a result of effective and timely diagnosis and treatment of malaria in the camp. As women who were parasitaemic during the study were more likely to be anaemic on admission than women who had no documented malaria, the authors concluded that they were probably infected before the start of the study, although randomization was performed correctly because anaemia was similar between those allocated to treatment or control. By treating all the malaria cases before the start of the study so that all cases seen were contracted during the study period may have reduced prior infection status to bias results, although this would have required a larger sample size and longer study period in order to observe any treatment effect. The study employed both active and passive case detection, which were well correlated. This demonstrates that among individuals with lower immunity to malaria and thus more likely to suffer symptoms, and where malaria screening and treatment was accessible, free passive case detection may be closely related to the actual malaria burden existing in the community and this method can be used as an effective malaria surveillance tool in the community. Under other conditions, e.g. where there are non-symptomatic malaria carriers or where health care is of low quality or costly to the user this may not be case. The principal vectors in this area are *Anopheles maculatus* and *Anopheles minimus*, vectors which exhibit a tendency to bite in the early evenings [64]. This vector behavior demonstrates a circumstance

where repellent use is beneficial, and the fact that no treatment effect was observed suggests that the sample size used was too small to observe the treatment effect, or that it may have been useful to use a higher concentration than 20% DEET to increase the duration of nightly protection. However, the major finding of the study was that there was no difference in the proportion of congenital abnormalities following the use of DEET between the treatment and control arms. Also no DEET was detected in the umbilical cord of 46 of 50 samples that were analyzed and none of the 30 samples of urine analyzed were found to contain more DEET than the acceptable levels of 0.1µg/ml. This study reaffirms that DEET is safe to use in the second and third trimesters of pregnancy [63].

In another household randomized, double blinded placebo-controlled trial recently conducted in Lao-PDR, to determine the effect of 15% DEET lotion topical repellent in addition to use of Permanet 2.0 LLINs on incidence of malaria did not demonstrate any intervention effect [65]. Field trials of 10-20% DEET carried out in the study area demonstrated a > 96% protection against all mosquito bites. The major malaria vectors in this region are the *Anopheles dirus* complex and *Anopheles minimus*, which are both outdoor and early evening biting vectors in the area [66], a characteristic which made repellent an ideal tool for control of malaria transmission in this setting. However, although the repellent was well received with over 90% of participants reporting that they liked using the lotions, compliance was still low with fewer than 60% of participants using the lotions more than 90% of the time. Focus group discussions (FGDs) revealed that the assumption that local populations were protected from night biting if they were provided with LLINs was not always true. Adult men and children reported spending time outdoors at night hunting and fishing and may have benefitted from using a longer-lasting repellent or even permethrin treated

clothing when engaging in night time outdoor activity. These behavioral factors, no doubt, increased bias and reduced the power of the study to detect an effect, if any. The treatment and placebo lotions both smelt and felt the same when applied on the skin and were presented in identical bottles, identifiable only by a three digit numerical code. Households were randomized to the treatments by drawing straws labeled with the codes of either repellent or placebo lotion. Follow up visits were done on random dates in order to ascertain compliance, and the field staff, data entry clerks and participants were blinded. However, it may have been possible for the participants to distinguish between the 2 treatments because placebo users were more likely to experience mosquito bites [67]. Treatments were administered at household level and to no more than 25% of households in any one village. This minimized the chances of treatment contamination, through diversion of mosquitoes from repellent to placebo users and confusion of treatments if individuals in the same household would have been issued with different treatments. However, this might have led to treatment contamination, which can occur through treatment non-adherence (not using the recommended intervention because of perceived lack of effect) and treatment crossover (receiving the intervention intended for the other group in a trial e.g. repellent users might give or sell their repellent to a neighbor). Both of these scenarios are common in repellent trials and create bias resulting in an underestimation or overestimation of the treatment effect in either arm of the study. In future trials this shortcoming can be addressed by using clusters of participants that do not interact with each other e.g. villages far apart, minimizing the chances of participants interacting with each other.

A study carried out in a forest fringe in India to determine the effect of 12% DEET used in conjunction with Insecticide treated mosquito nets (ITNs) on malaria

incidence demonstrated a protective efficacy of the intervention in the first and second year of the study respectively when compared to the control arm [68]. This study demonstrated a substantial effect of use of mosquito repellent and ITNs against malaria. The major malaria vectors in this area are *Anopheles dirus*, *Anopheles philippinensis* and *Anopheles minimus* which are generally early evening biting vectors [69], and so the repellents would protect against early evening biting which may explain why the repellents were additionally effective in reducing malaria among users of ITNs and repellents compared to ITNs only users. ITNs may confer communal protection by reducing vector populations [70], with additional protection from repellent use. This integrated management of vectors (IVM) using different tools (repellents and ITNs) would therefore have reduced vector populations and host parasite reservoirs by reducing human-vector contact, thereby lowering malaria transmission in the community. The study investigators collected baseline data on malaria incidence and vector bionomics before implementation of the intervention and were therefore able to establish the correct baseline incidence, reducing the chances of under-powering the study by using a smaller sample size. The study was also carried out for 2 years after 1 year of baseline data collection. This increased the sample size of the study, further minimizing the chances of under powering the study. The study had several positive features: it employed active case detection, minimizing the chances of missing malaria cases in the community making the estimation of treatment effect more robust. The research team also conducted random sniff checks to ascertain compliance to use of mosquito repellents and ITNs. Another aspect of this study that might have led to such a big treatment effect being observed is the promotion of the interventions through information, education and communication (IEC). For an intervention to be effective it has to be acceptable by the community.

Unlike other repellent studies, this study employed the use of IEC, which motivated the community to take up the intervention. This approach demonstrates that repellents can be an effective malaria control strategy if the community is well informed and educated and the intervention made available. Another finding of significance of this study is the further reduction of malaria incidence in the second year of the study implementation compared to the first year. This demonstrates that continuous implementation of an effective integrated vector management (IVM) tool can have a great impact on malaria transmission. However, the major shortcoming of this study is the paucity of information on how the findings were analyzed. This omission makes the findings questionable and surprising that the paper was published owing to the lack of information on even what method was used to analyze the data, the lack of data on slide positivity rates for the second and third years of the study and the highly questionable reliance on a converse interpretation of the risk ratio that was presented in the publication. The authors should have provided: 1) raw data on the number of cases per 100 man years per cluster or slide positivity rates in years two and three, 2) information on which model was used to analyze the findings, 3) why this model was preferred over other models 4) how the data was interpreted, 5) how bias was accounted for in order to make the findings credible to readers without having to rely upon the interpretation of the authors. The study as presented could not be used in a systematic review.

5.4.2 South America

A household randomized, double blind, placebo-controlled clinical trial conducted in Bolivia among users of a freshly impregnated ITN (25 mg/m² deltamethrin) plus either the insect repellent (*Corymbia maculata citriodon*) with a p-menthane 3,8 diol (PMD) concentration of 30% (MASTA, UK) for the treatment group or 0.1% clove

oil for the placebo group [67]. The study demonstrated an 80% reduction (IRR=0.2, 95% C.I. 0.11 – 0.38, $P < 0.001$) in *Plasmodium vivax* malaria. However the effect on *Plasmodium falciparum* malaria was not significant most likely due to lack of power as the number of *Plasmodium falciparum* cases was too low to demonstrate any treatment effect. This might be because of an unexpected round of fogging as explained by the authors, but they also offer the more likely explanation that the study took place when transmission of *Plasmodium falciparum* was low. Sequential randomization of households was used to allocate treatments and both the participants and field staff were blinded. Both these attributes increased the robustness of the study, as there was a minimal chance of selection bias by the field staff or the participants not using the placebo. The use of a clove oil repellent was useful in this circumstance as both PMD and clove oil have a strong odour, which would suggest to the users that both were active repellents. However, there was always the chance of the control group realizing that they were issued with the placebo as the trial went on and dropping out of the study, thereby reducing the power the study because of decreased sample size. The study took place for only 4 months and thus effect of repellent over the whole malaria transmission period could not be determined. Had the study been conducted for longer to take into account the whole transmission season, then a treatment effect is more likely to have been observed against *Plasmodium falciparum* malaria, or even a larger, more robust estimate of treatment effect observed as the sample size would have been larger consequently reducing sampling error and improving effect estimates. The major vector found in this region, *Anopheles darlingi*, has a peak biting time from 8 - 10p.m. [71] and is strongly exophagic and endophillic [72], and therefore it is recommended that repellents be used at this time as people are not under their LLINs. PMD is extremely effective

against even high densities of local malaria vectors and is likely to have provided users relief from high densities of mosquitoes during the wet season [73]. Overall, the study demonstrates that mosquito repellent used in the early evening in conjunction with LLINs in regions of early evening vector biting can impact on malaria incidence, strengthening the case for employment of IVM in malaria control. The compliance of the study participants was reported to be very high, underlined by their preference for PMD measured by focus groups [74] and this was confirmed by random sniff checks by the field staff. The large treatment effect observed was likely a combination of a well designed and implemented trial methodology conducted in an area where vector bionomics preclude control by other means and where the repellent was well complied with because it was both highly effective against mosquitoes and cosmetically acceptable to the local population.

5.4.3 Sub-Saharan Africa

In a cluster randomized controlled trial conducted in Ethiopia, to determine the effect of Buzz Off repellent on malaria, the odds of contracting malaria was reduced by 43% (OR=0.57, 95% CI 0.35-0.94, P = 0.028) for the participants using repellents to supplement Permanet 2.0 LLINs [75]. In this study, data was collected by 3 cross-sectional surveys during the 4-month study. It would have been more prudent for the study investigators to conduct the study throughout the year so as to take into account the whole malaria transmission season and during the wet and dry seasons. This would have produced a more realistic estimate of malaria in this region. It would also have increased the sample size of the study thereby decreasing the chances of a type II error. Also, some cases of malaria may have been omitted as data was collected for only part of the transmission season. The authors of this trial did not also outline the active ingredient (AI) and amount present in the repellent. Information on how

randomization was conducted was missing. Although there was good similarity of socioeconomic variables between the treatment arms, randomization could not have been performed correctly because at baseline the 2 treatment groups were not similar in terms of malaria prevalence – there was twice as much malaria in the repellent arm of the trial, the control arm complied with and had more LLINs, and two of the eight clusters had been sprayed with DDT (to which arm of the study these were allocated is not stated) and this might have confounded the results of the trial. This resulted in the investigators altering the analysis plan of the study. When the authors followed the analysis plan outlined in their protocol there was no difference seen between treatment arms. As a consequence, the authors changed their analysis, which might have altered the treatment effect observed since the data was not designed to be analyzed in this way.

A double blind, placebo-controlled cluster-randomized trial of 15% DEET topical repellent carried out in south-west Tanzania, demonstrated a non-significant protective effect of 27% reduction in household malaria rates from 91.17 cases/1000 person years [95% C.I. 198.42 – 380.76] in the control arm to 65.37 cases/1000 person years [95% C.I. 110.10 – 240.84] in the intervention arm, ($p=0.40$, $z=0.84$) using intention to treat analysis [76, 77]. These findings were however not significant, possibly because the study was underpowered. The major vector in this area is *Anopheles arabiensis*, which bites both indoors and outdoors from 6 am to 6 pm and it was estimated that a repellent could reduce around 30% of exposure based on the average time to bed of 9 pm. Both semi-field and field evaluations of the efficacy of 15% DEET repellent, demonstrated > 80% protection against mosquito bites for four hours against *Anopheles arabiensis* mosquitoes. However, effectiveness of an intervention is a component of both efficacy and acceptability of that intervention by

the community. Therefore, to ensure effectiveness, the study team conducted 3 rounds of social marketing of the repellent in the study area to encourage uptake. This had positive results as uptake was reported at 95%. However, despite all these checks put in place during project implementation, a treatment effect was still not observed. This was mainly due to two reasons; first, the study team overestimated the baseline malaria incidence by extrapolating incidence from all cause fever data and therefore estimated a sample size smaller than was needed to observe a treatment effect. Second, a drought that had occurred during the study period, lowered malaria transmission such that a treatment effect could not be observed. In future studies, it would be useful to conduct baseline malaria incidence studies so as to establish the correct incidence estimates for sample size calculation. Compliance was determined by self-reporting, which was done at the end of every month when field workers visited the households to issue new bottles of repellent/placebo. Therefore compliance in-between the monthly visits could not be ascertained. However a few random sniff checks were conducted and these spot checks ascertained that the participants did indeed use the treatments issued. It would, however, been practical to conduct the checks every fortnight and compare them with self-reported compliance so as to establish a correlation between the two methods of determining compliance. Passive case detection of malaria by Rapid diagnostic tests (RDTs) was employed at the local dispensary where participants were offered free diagnosis and treatment. People did not believe the results of negative RDTs and some stopped attending the dispensary, preferring to self medicate with anti-malarial drugs or attend the other health facility in the village that used clinical diagnosis. Also, the health dispensary recruited into the trial may have been sufficiently far from the homes of some participants to prompt them to access alternative health facilities or go to a nearby drugstore. In future it

would be useful to recruit all health facilities and drug stores in the study area to avoid loss of malaria cases and to carry out active case detection. All these factors might have contributed to reduction of malaria cases, lowering the sample size, thereby under powering the study. The randomization of interventions and blinding was done as effectively as possible for this case by using treatment and placebo lotions in identical bottles identifiable only by a three-digit code. Even then, as time went by, participants realized that they were issued with a placebo because they were continuously being bitten. As result there might have been some treatment contamination where placebo users did not use their intervention and repellent users sold their repellents to their neighbors lowering the power of observing a treatment effect. It was also suspected that study participants gave their ID cards to relatives and friends to benefit from free health care. This would also lead to treatment contamination that could be overcome with the use of a fingerprint scanner or photographic ID to identify study participants.

A field clinical trial conducted in Isfahan, Iran, to determine the effectiveness of DEET sticks against Leishmaniasis, in 430 students (50% male, 50% female) did not demonstrate any treatment effect [78]. The intervention was a DEET stick reported as effective for 18-20 hours and its minimum effective concentration was 55-77 $\mu\text{g}/\text{cm}^2$. DEET was randomized to 330 individuals and placebo stick was randomised to 100 controls and the treatment allocation code of sticks was revealed only at the end of study. The children were followed up for 10 months. The efficacy of these sticks were evaluated in terms of reduction in infection by leishmaniasis using relative risk (RR). Confusingly, in the results section, the study reported a different number of treatments and controls: out of 200 students that were protected using placebo pen, 2 students

acquired leishmaniasis and out of 230 students that were protected using DEET pen, 8 students acquired leishmaniasis. Thus the study cannot be accurately interpreted.

5.5 Case control studies

Apart from RCTs, there have been case control studies that have been conducted to evaluate the impact of repellents on disease. Case controls are observational studies of persons with disease and a suitable control group of persons without the disease, where a potential risk factor is examined by comparing the frequency of occurrence of the risk factor between these two groups [79]. There have been a number of case control studies that have been conducted to determine the effect of repellents on malaria incidence.

In Afghanistan, a case control study was conducted through social marketing of Mosbar, a repellent soap containing 20% DEET and 0.5% permethrin [80]. Cases and controls were recruited through passive case detection at the local clinic. The combined use of Mosbar and ITNs demonstrated a 69% reduction in the odds of contracting malaria (O.R.= 0.31, 0.13 - 0.72, P = 0.007), when compared to control (neither Mosbar nor ITN). The local mosquito vectors, *Anopheles stephensi* and *Anopheles nigerimmus* bite shortly after dusk, and through out the night, a characteristic that makes repellents a suitable control tool for early evening protection before LLINs can be employed. The repellent selected was highly efficacious and gave 100% protection for the whole night, which might have promoted the observation of the treatment effect. However as a hospital based case-control, this study was prone to selection bias and therefore could not be generalized to the rest of the population, as individuals attending the clinics recruited into the trial might have had different characteristics from individuals in the general population [80].

There are a number of anecdotal case control studies that were not specially designed to measure the effect of repellents as the above study, but to identify risk factors among those with malaria.

In a case control study of risk factors among British travellers returning from the Gambia, less use of repellents was associated with a greater risk of contracting malaria [81]. The use of repellents, applied either on the skin or on clothes, is a key strategy of bite avoidance recommended in travel medicine. This finding illustrates the importance of the use of repellents when travelling to malaria endemic regions. All individuals travelling to malaria prone areas should therefore be advised to employ malaria control strategies to protect against malaria. Also, tourist destinations should provide information on the vectors that are present in these regions so that the tourist can be better advised and prepared on which tools to employ. It also emphasizes the importance of having international guidelines for travellers visiting malaria endemic regions to avoid importing malaria cases to their mother countries.

In Kilifi Kenya in a large, (>1,500 participants), well-designed case control study, use of local repellents, mosquito coils and insecticide sprays was significantly associated with protection from developing severe malaria after adjusting for confounders (OR= 0.57, 95% CI 0.35-0.94, p=0.02). The cases and the controls were chosen from the same area in the community. Consequently, these results could also not be generalized to the whole population, as the individuals from this area of the community might be different from the other members of the community. It would have been better to select more than one study area to make the findings more general to the population [82]. A study from the Gambia using almost identical design to the study in Kenya showed an association with the use of coils in preventing severe

malaria in a univariate analysis but this association was not observed after multiple logistic regression [83].

The overall evidence generated by the above studies demonstrated that use of repellents can be effective against malaria transmission if these interventions are used correctly and with sufficient frequency. In studies where an association cannot be established it is usually because of poor study design. The following series of studies are inconclusive due to a number of factors including poor matching; poor attention to sample size; and poor measurement of compliance, which is the single most important factor in the effectiveness of any repellent.

In another case study in India, individuals who did not use repellents had non-statistically significant lower odds of malaria, (O.R.= 0.85, 95% C.I. 0.57 – 1.28, P = 0.41) compared to those who used repellents. This finding is not consistent with other repellent trials and there are various factors that might have led to this conclusion, especially as those exposed to higher levels of mosquito bites are more likely to use mosquito prevention tools. In addition, the cases and controls were not matched because the controls were recruited from the same clinic, assumed to have come from the same socio-economic, demographic and geographical area as cases. Because of the study design there was no way to establish compliance to repellent use. Also longevity and quality of these repellents could not be established although the mosquito coils and mats were reported to be allethrins and topical repellents used were diethyl toluamide (DEET) – for which the concentration was not mentioned. The bionomics of the local vectors was not discussed to determine whether use of repellents would be an appropriate tool [84].

Similarly in another case – control study in Burkina Faso, use of mosquito coils and burning smoke (spatial repellents) was not associated with a lower risk of malaria;

(O.R.=1.24, 95% C.I. 0.73 – 2.00, P = 0.47) and (O.R= 0.74, 95% C.I. 0.35 - 1.56, P = 0.43) in the treatment and control groups respectively. Like the aforementioned study, use of mosquito coils and burning of plant leaves for smoke were self-reported. The study participants might have over reported or under reported compliance, biasing the findings on the study. The controls were recruited from the same residential area. As a result, these findings cannot be generalized to the whole population, as the individuals from this area might not have similar characteristic to the general population. The controls from this study were not actively tested for malaria and were assumed to be malaria negative. This might have biased the study toward the null hypothesis if the controls were positive for malaria [85].

In Ecuador and Peru, a community randomized trial of Mosbar, a mosquito-repellent soap containing 20% DEET and 0.5% permethrin did not show any significant reduction in malaria incidence between the intervention and control groups [86]. The effect of the repellent soap was studied under different settings. It was found to be efficacious only when individuals wearing the soap were inactive after application. This contrasts with the findings from Pakistan [61] where the repellent was extremely effective in preventing mosquito bites and this might be due to the higher relative humidity in the Ecuadorian site, that caused more rapid loss of repellent through sweating. Compliance to repellent use was not established and lack of treatment effect may have been due to poor compliance, as many people did not like the smell of the repellent, and in Ecuador, because of humidity, a thick layer of soap remained on the skin, which was not pleasing to the users. As compliance requires high degree of motivation, it was necessary for the study team to socially market their intervention to encourage use and user acceptability. Interestingly, user compliance was drastically reduced when the soap was only made available from shops and was no longer

available free of charge. This was similar to findings in other studies and underscores the importance of developing low cost or highly subsidized interventions that can be accessed by those of low-socio-economic status in disease endemic countries and who are also most at risk from disease morbidity and mortality. For an intervention to be effective it has to be acceptable to the community, affordable or free to the community.

5.6 Cross sectional studies

Cross-sectional studies are research methods that involve observation of all of a population or a representative subset at a specific point in time. They collect data on outcomes and/or exposures from each participant at one moment in time. Thus while they are simple and quick to perform they are more robust at measuring associations with chronic diseases because they measure prevalent rather than incident outcomes. Cross sectional studies that collect data on both outcome and exposure are not very robust in establishing causal effect of an intervention, as they are prone to bias from confounding factors, but they can be used to test hypotheses about interventions and to justify a research objective.

A cross-sectional survey was carried out in the Thai-Myanmar border in Northern Thailand to determine risk factors that contribute to malaria infection. Malaria prevalence was extremely high in 46% of participants. It was a well designed study that had correctly used sample size calculation and demonstrated a clear relationship between working or staying overnight in the forest and having malaria in univariate and multivariate analysis, while use of topical repellents and long clothing was protective against contracting malaria on univariate analysis, but this treatment effect was not seen when all confounders were taken into account. This study shows some of the practical scenarios where topical repellents can be employed, like individuals

working in the forest or in the crop fields who are not able to employ the conventional control measures like LLINs [87].

A cross-sectional survey to determine the effect of Personal Protective Measures (PPMs) against malaria in travellers, demonstrated a significant reduction in malaria among travellers who used protective clothing, covering their arms and legs.

However, no significant reduction was associated with use of repellents and coils. As explained in this study, compliance to PPMs was very poor with a large proportion of the study participants. This would likely explain the lack of treatment effect. Also, it is advisable that more stringent measures by the responsible agencies be introduced to ensure compliance to PPMs by people travelling to malaria endemic regions to avoid exposing non immune individuals to malaria and also reduce importation of cases to their mother countries [88]. Compliance to personal protection is surprisingly low among those with access to the correct preventative measures. A recent survey among 2,205 individuals from the French Military during and after their stay in malaria-endemic areas, demonstrated a malaria incidence of 2.98 cases per 100 subject-years [89]. The “correct” compliance rates were 48.6% (95% CI: 46.5 – 50.7%); ranging from 2.6% to 88.2%, 50.6% (95% CI: 48.5 – 52.7%); ranging from 1.7% to 97.3% and 18.5% (95% CI: 16.8 – 20.1%); ranging from 4.9% to 59.6%; for wearing long clothing at night; using LLIN while sleeping and using insect repellents, respectively. Factors that often influenced compliance were gender, the rainy season, mosquito bite burden, and perceived mosquito attractiveness compared with other people, while perception of the severity of malaria was not associated with regular use of any of the methods measured. In a further cross sectional survey of 89,617 travellers returning from East Africa between 1988 and 1991 [88], only 2% of respondents stated that they regularly complied with air-conditioned rooms and/or bed nets, adequate

clothing, and use of insecticides and/or coils. Regular use of personal protection resulted in a small, but significant reduction of malaria incidence when travellers were interviewed 12 weeks after returning home, but each method alone showed no significant effect. Unlike the situation among the French military, the holiday makers increased their compliance in periods when more mosquito bites were noticed, but similar to the French study, gender had no significant influence on compliance, and surprisingly, neither did diagnosed or suspected pregnancy. Those using no chemoprophylaxis were not more vigilant in preventing mosquito bites. Compliance diminished continuously with length of stay in Africa: among those who stayed up to 2 weeks, the compliance rate was 77.2%, while in those staying 2 months or more, the rate was 63.3% ($p < 0.001$).

5.7 Outbreak reports

In South Africa, topical application of 15% DEET to feet and ankles reduced overall *Anopheles arabiensis* bites by 69% in field observations. This led to testing of this intervention under operational conditions during a malaria outbreak in Mpumalanga, 15kms South of the Kruger national park. The implementation of the intervention was associated with an immediate drop in malaria incidence from 42 cases per week to 10 cases per week. This effect is however difficult to interpret as it could have been due to the repellent but it may also be due to the fact that the epidemic curve had peaked and was dropping naturally. The repellent may however have helped in maintaining the low incidence of malaria. This study does however give situations where repellents can be employed. The most likely reason why the more effective LLINs were not used in this particular scenario is that the major vector in this area, *Anopheles arabiensis* had behaviourally adapted to outdoor biting and the secondary vector, *Anopheles funestus* had developed resistance to IRS [90]. Although the results

are not clear, this study represents a useful scenario in which repellents might be employed against malaria.

In an outbreak report to describe the outbreak of *P.vivax* malaria in Far North Queensland, Australia, individuals who used topical repellents (DEET) were at 0.01 (95% C.I. 0.00 – 0.19) the odds of developing malaria when compared to non-repellent users. The findings of this study reinforce the need to use other personal protection measures in areas where conventional malaria control tools are not applicable [91].

During an outbreak in India, a well-designed investigation was conducted where malaria cases were slide-confirmed and compared with matched neighborhood controls. For both groups, information on personal protection use was collected using questionnaires and data was compared using matched odds ratios (MORs) [92]. In total, 7,303 cases and 17 deaths were reported between April 2005 and March 2006 with a peak during October rains (Attack rate: 50 per 1,000, case fatality: 0.2%), and half of the cases were detected by active case detection. Use of repellents was associated with an odds of 0.1 (95% C.I. 0.06-0.3) of contracting malaria and failure to use repellents was associated with 69% of malaria cases in the population.

Compared with controls, cases were more likely to sleep outdoors (MOR: 3.8, 95% CI 2.2 - 6.5) and less likely to use mosquito nets and repellents (MOR: 0.3, 95% CI 0.1 - 0.5). In this outbreak investigation, the villagers reported the use of repellents and coils and therefore correct and consistent compliance could not be established. This might have biased the treatment effect seen. Also, being a retrospective case-control, this study might have been prone to recall bias. Despite these shortcomings, this study demonstrated a protective trend of mosquito repellents against malaria.

There are a large number of disease outbreak reports among military personnel related to non-compliance with the standard personal protection measures (PPMs) [93]. A report from the French army monitoring leishmaniasis among troops stationed in Guinea demonstrated four separate outbreaks of leishmaniasis in which troops admitted that they did not use personal protection correctly [94]. In a malaria outbreak in French Guiana, a retrospective cohort study found that malaria was associated with low compliance to impregnated battle dress uniforms (BDUs) [95]. This study also brings to the fore the problem of compliance to repellent use. As studies mentioned earlier have shown, for repellents to be effective, then they must be acceptable to the individuals to which they have been issued and they must be used correctly and consistently. Similarly, in a malaria outbreak in Sierra Leone among British soldiers, a case control study demonstrated that use of insecticide treated clothing offered significant protection against malaria with almost 50% fewer cases being reported among those individuals who used their impregnated battle dress (OR=0.57, 95% CI 0.20 – 1.05 p = 0.0450. Interestingly, the use of multiple protective measures gave even better protection (OR=0.29 95% CI 0.10 - 0.80 p=0.007). However, use of repellents and chemoprophylaxis showed no significant effect [96]. In a malaria outbreak in 2003, 44 U.S. Marines were evacuated from Liberia with either confirmed or presumed *Plasmodium falciparum* malaria [97]. An outbreak investigation showed that only 19 (45%) used insect repellent, 5 (12%) used permethrin- treated clothing, and none used bed netting, demonstrating further the importance of compliance in personal protection from vector borne disease.

5.8 Permethrin-treated clothing evaluation: Randomized controlled trials

5.8.1 Southern and South East Asia

In Afghanistan, a randomized controlled trial of 1g/m² permethrin-impregnated *chaddars* (cloth used as a head covering [and veil and shawl] by Muslim and Hindu women) reduced the odds of having *P. falciparum* and *P. vivax* malaria by 64%, (O.R.= 0.36, 95% C.I. 0.20, P = 0.001) and 38%, (O.R.=0.62. 95% C.I. 0.36 – 1.06, P = 0.069), respectively. There was a significant effect in the 0-10 year and 10 – 20 year age group. This trial, however, showed no effect on malaria incidence in refugees > 20 years of age [98]. In this study, no information was given on how the [98] randomization was carried out. The trial took place over 5 months and therefore did not capture the effect of repellents over the entire malaria transmission season. Since the study was carried out at the end of *P. vivax* transmission season and the start of the *P. falciparum* season, this might explain why there was a larger treatment effect seen on *P. falciparum* transmission compared to *P. vivax* transmission. It is possible that, had the study been carried out for longer, then a larger effect would have been observed. As *P. vivax* malaria is known to recrudescence, the study investigators should have cleared all malaria cases through an appropriate treatment regimen after checking for glucose-6-phosphate dehydrogenase (G6PD) deficient individuals [62], so that any cases that were observed would be classified as new malaria cases and not recurrent *P. vivax* cases. The study employed passive surveillance of malaria cases; consequently some cases not reporting to the health clinic might have been missed, lowering the sample size and power of the study to observe a treatment effect. This might explain why a treatment effect was not seen among females, because they were less likely to leave the home due to the practice of *purdah*. In the evening they might also have been using their *chaddars* as bedding for their children as a protective effect

was only seen among those individuals less than 20 years of age. Compliance was established by visiting the households every 2 months. As frequent compliance inspection was not done, compliance in between the months cannot be ascertained, and hence the findings of the study are less robust. As with all intervention studies, compliance is essential for the intervention to be considered effective, although the *chaddar* is a piece of clothing used on a daily basis.

A second single blind RCT by the same group, that investigated the effect of insecticide-treated nets (ITNs), insecticide-treated *chaddars* used to sleep in, and residual pyrethroid spraying of individual houses for the prevention of cutaneous leishmaniasis (CL) in Kabul, Afghanistan, also demonstrated a significant protective effect [99]. The incidence of CL among those randomized to the control was 7.2%, amongst ITN users, 2.4% (OR 0.31, 95% CI 0.2-0.5), amongst impregnated *chaddar* users, 2.5% (OR 0.33, 95% CI 0.2-0.6), even better than that among those living in lambda-cyhalothrin sprayed houses 4.4% (OR 0.60, 95% CI 0.3-0.95). ITNs and impregnated *chaddars* were equally effective, providing about 65% protective efficacy, with approximately 40% protective efficacy attributable to individual house spraying. The study was well powered: it was conducted in 1997/98 amongst a non-immune population of 3666 people over 15 months. New cases of CL were diagnosed based upon clinical inspection of lesions, but parasitological confirmation could not be completed after aid organizations were ejected from Kabul in July 1988. Another difficulty of working in such a challenging environment was that compliance could not be measured, because spot-checking would have invaded privacy customs strongly upheld in the region. No significant differences for age or sex were found between new cases in the intervention and control groups. No serious side effects were reported and interventions were generally popular; ITNs were the most popular,

followed by residual spraying and then impregnated *chaddars*. Both ITNs and *chaddars* are useful in this region, as the population tends to be quite mobile. This population mobility caused massive loss to follow up (45%) as people moved out of the study area, but the study investigators had anticipated this and accounted for it during recruitment of study participants. This demonstrates the importance of recruiting the appropriate sample size in any study.

A double blind, placebo-controlled trial to determine the efficacy of permethrin-impregnated uniforms among Iranian soldiers, in Isfahan, Iran, demonstrated a reduction in the odds of contracting Cutaneous Leishmaniasis (C.L.). However this effect was not significant, possibly because the study had only 134 people per treatment arm for 3 months exposure in the field (1,608 person weeks per arm).

Compliance was high, as the soldiers were required to wear the uniforms day and night and was monitored. As compliance was ascertained, the results of this study may be credible. The method used for randomization was however not described.

This may have been done incorrectly, biasing the study and hence the observation of no treatment effect in the treatment arm. Both the participants and study investigators were blinded to the treatments, reducing chances of selection bias [100]. The study shows that permethrin impregnated uniforms are safe for human use and no adverse effects were observed. Therefore, they present a potential tool that can be explored for malaria control. Of importance is to note is the fact that all the lesions (sites of infection) among the treated uniform group were on sites unprotected by the uniform (face and wrist), while in the control group the lesions were found on the arm [100] and trunk. If the soldiers had been using full personal protection including a topical repellent on their face and hands [14], they may not have contracted leishmaniasis.

In the Thailand-Cambodia border, a randomized placebo-controlled trial evaluating the effect of 2g permethrin per uniform on preventing malaria versus kerosene treated uniforms among the Royal Thai army demonstrated no effect. The population was 403 male soldiers on active duty for 6 months. The randomization method was not outlined in this study and compliance could not be established at all times. Both these factors could have confounded the findings of this study as the selected study participants might have confounding characteristics. Also, as compliance could not be established, both groups might not have used the repellent, therefore biasing the study towards the null. One study arm may also not have complied with the intervention similarly driving the effect towards the null [101].

5.8.2 South America

A double blinded placebo controlled study in Colombia among 86 soldiers randomized to 600-712mg/m² permethrin treated uniforms and 86 soldiers randomized to water-treated uniforms over 4.2 weeks showed the uniforms to be 79% protective against malaria, 3% versus 14% among treated and control group, respectively and 75% protective against cutaneous leishmaniasis 3% versus 12% among treated and control group, respectively [102].

The same double blind, RCT carried out in Colombia to determine the efficacy of permethrin-impregnated uniforms against both malaria and CL demonstrated a reduction in relative risk of malaria (RR=0.29, p=0.015) and CL (RR=0.21, p=0.002) [102]. As adherence to instructions to wear the permethrin treated clothing day and night could not be monitored, the findings of this study might be debatable as with all studies where compliance could not be established. However, the monitoring of disease was actively done everyday and it's unlikely that any cases of malaria or CL could have been missed. There were very few reports of adverse effect of insecticide

treated clothing. This is similar to the other studies where very few adverse effects have been reported; reinforcing the proposition that insecticide-impregnated clothing is safe for human use. This intervention can be applied to normal clothing thereby tackling the problem of adherence so often seen when using topical repellents.

5.8.3 Sub-Saharan Africa

In a randomized community trial, 198 Somali refugees of all ages and both genders with no known allergies or respiratory problems at the Dadaab refugee camp, were randomised to either 0.37% permethrin treated clothing and bedding or water placebo (retreated every three weeks) for a period of three months. All clothing and bedding were treated including, *Diras, Saris, Jalbaabs, Ma'awis*, shirts sheets and blankets. Use of the permethrin treated clothing and bedding significantly reduced the odds of contracting malaria by 70% (CI not reported) [103]. Methods for randomizing treatments was described as systematic random sampling of households within treatment and control blocks 1.5 km apart, and compliance was maintained by regular retreatment of all clothing and bedding. The participants and laboratory technicians were blinded to the treatments. These aspects of the design are positive. However, the study was small and the statistical reporting was not good as it was unclear, over-reliant on models and *p* values and OR's were reported without confidence intervals. However, the study reported the proportion positive in the intervention and control group and the number of people tested, and therefore this data could be used for a meta-analysis.

In another randomized community trial in Kenya to determine the effect of appropriate permethrin impregnated clothing against malaria, it was found that the incidence rate ratio (IRR) of contracting malaria in those aged over 5 years in the intervention group was (IRR=0.187, 95% C.I. 0.046-0.770, *p* = 0.02) compared to the

control group [104]. For those under 5 years of age, however, no effect was seen. A total of 472 individuals were enrolled in the randomized community trial where the unit of randomization was the hamlet (*manyatta*) with 234 and 238 in the intervention and control arms, respectively. Baseline data included socio-demographic data, parasite prevalence data from thick and thin blood smears, and clinical measures of malaria. The intervention involved the dipping of *shukas* owned by the intervention group in permethrin although the dose was not available in the publication. The prevalence of malaria in the study population (based on slide confirmation) was considerably lower than that used for the power calculation based on clinical estimates (2.2% versus 20%). For those aged 6 years or over, the rate of malaria cases (events per 10 000 person-days at risk) was 1.41 in the experimental group versus 7.49 in the control group (IRR=0.187, 95% CI: 0.046– 0.770). For children less than 5 years of age, results were imprecise with no clear benefit of the intervention. An attempt was made to impregnate all *shukas* in the experimental group. However, some children refused to have their *shukas* dipped in the cold early morning hours, as it was their only clothing. Other children, one-third of the 5 years and under in both groups owned no *shuka*. The researchers had been aware of this prior to the study but had felt that this should not affect results since preliminary research indicated that children without *shukas* slept under their mother's *shuka* at night. Of the four cases that occurred in the intervention group, three did not own their own *shuka* and the fourth owned a *shuka* that was not impregnated. This incomplete coverage, coupled with the fact that the study investigators did not establish the local baseline incidence rate led to an underestimation of the sample size required to observe a treatment effect. This shortcoming underlines the importance of establishing baseline factors before any study is implemented. Clinic reports implied that 35% of all patients were seen for

malaria, and the clinicians' predicted prevalence of parasitemia was even higher (50%). Although a more conservative 20% was used to calculate sample size, the 2.2% parasitemia observed at baseline clearly reduced the statistical power of the study. This highlights the unreliability of malaria reports based on clinical diagnoses, which was also one of the reasons for the Tanzanian study of DEET repellent being underpowered.

5.9 Other studies

In a clinical trial in Myanmar, the use of treated scarves and hand-bands were significantly associated with lower incidence of malaria compared to the control arm where these interventions were not employed [105]. The major local vector is *Anopheles minimus*, an outdoor and early evening biting vector. This makes treated scarves and hand bands an appropriate control tool in this setting, as conventional tools cannot be employed at these places and times. The study was carried out for a short period of time and did not take into account the low transmission season and it was therefore not possible to establish the seasonal effect of this intervention.

Compliance assessment was carried out in 10% of the study participants. From this sample the compliance of the entire study population could be inferred. Also the investigators carried out bimonthly checks on compliance and random spot checks. The compliance monitoring of this study was well conducted and the results can be considered credible. The results from toxicity evaluations of this intervention did not demonstrate any adverse effect. This was in agreement with other studies that assessed the toxicity of insecticide treated clothing.

All the earlier mentioned studies are associated with a protective trend of repellents against malaria. Most studies had questionable study designs and therefore the results of these studies could not be conclusively relied upon. However, the fact that a

protective trend was observed in all of them reinforces the need to conduct a well-designed large-scale trial to ascertain the effect of repellents on disease incidence.

5.10 Mosquito coils: Randomised controlled trials in South-east Asia

There have been 2 randomized trials that have evaluated the impact of burning mosquito coils every evening on malaria transmission, both conducted in Southeast Asia. The first study [67] was a single blind, cluster-randomised, comparative control clinical trial conducted in Ruili district, Yunnan Province, Peoples Republic of China, close to the Myanmar border between April and October 2007. Yunnan is one of only two provinces in China that still has malaria cases and Ruili district has a particularly high number of cases. The area is heavily forested, with a high proportion of migrant populations moving over the border between countries and has many remotely located minority group habitations, making implementation of vector control and public health programs extremely difficult. All communities enrolled were in rural areas. The trial was designed to measure and compare the protection against malaria provided by mosquito coils, LLIN or a combination of the two. The study recruited 2,052 households comprising 7,341 individuals, excluding individuals under 6 years and pregnant women. Households were randomized to one of four groups; Coils (0.03% transfluthrin coils, SC Johnson), deltamethrin LLIN (TianJin-Yorkool Ltd, Tianjian, PR China, and Lantrade Global Supplies Ltd, Gerrards Cross, UK), coil plus LLINs and a control group without intervention than whatever control intervention they were already using. At baseline, and every month post intervention, each individual was actively screened for malaria (both *P. falciparum* and *P. vivax*) by RDT. At the end of the 6-month study there were 69 confirmed malaria cases in the control group, 16 in coil group, 14 in the LLIN group and 5 in combined coil plus LLIN group. In the coils only group the age adjusted odds ratio for *P. falciparum*

malaria was (OR=0.23, 95% CI 0.11-0.50 $p = 0.0002$) and protective efficacy against *P. vivax* was 80%, (O.R =0.20, 95% CI 0.09 -0.44, $p<0.0001$) and was not significantly different to that for LLINs or LLINs plus coils. The level of compliance with the allocated intervention was high: > 94% of individuals used the coils and/or LLINs for > 90% of the month prior to the surveys. Conversely, those in the control arm were less compliant, with 13-19% using local coils for 3 or more days per month. According to protocol analysis including only those with > 90% compliance gave almost identical results to the intention-to-treat analysis.

A second, more recent double blind, placebo controlled cluster randomized trial in Sumba, Indonesia, to evaluate 0.0097% metofluthrin mosquito coils only (no LLINs were used in either study arm) against malaria [106] comprised two clusters (1000 people) allocated to treatment arm and two clusters (1000 people) allocated to control arm. Of these, 45 Healthy males > 17 years, >40 kg, G6PD normal residents in the study village for the study period in the two clusters per arm (n=90 per arm) were followed up as the study cohort for 26 weeks. Compliance was monitored daily and active case detection was monitored weekly in addition to malaria vector abundance by location, distance and time by indoor and outdoor human landing catch and larval collection, parity rates by detinova ovarian dissections, and sporozoite rate by ELISA. Malaria incidence among the treatment group was 0.904 versus 2.324, which equates to a 61.1% protective efficacy (95%CI =37%-75%, $p<0.00001$).

5.11 Conclusions

These two trials of spatially acting pyrethroids used as mosquito coils were tested in isolation – without the addition of LLINs and provided unambiguous evidence that individual malaria risk is significantly reduced by > 60% simply through avoiding mosquito bites. These trials were conducted under rigorous conditions that should set

the bench mark for future trials because they were designed, powered and analyzed with the help of a statistician; had adequate randomization; were placebo controlled allowing adequate blinding (Sumba study) and used active case detection with RDT and PCR confirmation throughout the study. In addition, essential to the success of any repellent intervention, very high compliance was observed throughout the study, carefully monitored by study staff. Furthermore, both studies were conducted in suitable field sites. In both cases, a large proportion of mosquito bites occurred before bedtime (Table 4) and mosquito coils were culturally acceptable (a smoky environment is tolerated). In addition, repellents may be more effective in Southeast Asia because malaria transmission is low and seasonal and the main malaria vectors are opportunistic and will feed on other hosts.

Future trials should attempt to match the high standards of these trials and also include some further information on community level measurements of the impact of mosquito coils on malaria vector population dynamics. These data were collected in some extremely detailed studies on dichlorvos during the 1960s and demonstrated that at high enough coverage of repellent interventions there can be a community protection demonstrated by decreased man-vector contact, vector infectiousness and vector longevity.

This is the key piece of information that should be collected from any future trials of personal protection tools if they are ever to be considered as public health tools applied at a community scale to prevent disease transmitted outdoors, in the day or evening, rather than just niche tools for particular lifestyles or occupations.

Furthermore, dichlorvos is an example of a repellent tool that required little compliance – just replacement of dispensers every two weeks. It is essential that

future research looks into such low compliance interventions that will help address the two greatest barriers to repellent implementation: cost and compliance.

Findings from the review strongly support the theory that use of repellents has a beneficial protective effect against transmission of disease, mainly, malaria and leishmania, as there is very little data available on dengue. Even though individual studies had varying outcomes, the combination of all the available evidence does support the notion that specific repellents should be incorporated into current vector control strategies where appropriate. We recommend the use of repellents (both spatial and topical) for use at times when current control measures cannot be implemented. The other key finding from this review was the paucity of high quality data that exists. In order to improve the speed at which products are developed and approved by bodies such as WHO, there is a clear need for harmonization of methodologies and outcomes measured in new trials for evaluation of vector control tools, in particular the way they are reported. Researchers need to be encouraged to see their piece of research as a contribution to the overall picture in a research field. Clear reporting of outcomes and use of guidance available for this task e.g. using CONSORT guidelines [107] should make future trials more robust and data easier to assimilate by means such as systematic review and meta-analysis for use by policy makers. It was also clear from this review that those trials collecting data through active case detection were far more powerful than those employing passive case detection because the latter was prone to bias. Important secondary endpoints of any trial are entomological correlates of reduced infection i.e. man-vector contact, parity rate, sporozoite rate through regular human landing catches and human compliance with the intervention. An exposure free measurement of human landing is especially needed for large-scale epidemiological work especially in areas where Dengue or

other arboviruses are prevalent. Measurements of compliance such as salivary antigen markers of exposure to mosquito bites [108] are a key research need for rigorous and ethical research into disease prevention using vector control tools as it may be used as both a measure of exposure and compliance.

Table 5:1 Summary of evidence demonstrating that repellency can have an effect at a community level by reducing vector survival. Data is for Dichlorvos - a spatially active organochloride - that is no longer licensed for this purpose

Study	Region	Vector	Concentration	Duration	Mosquito density	Mosquito biting activity	Place of biting	Sporozoite rate	Malaria prevalence (before)	Malaria prevalence (after)
[109, 110]	Haiti	<i>Anopheles albimanus</i>	25% in Wax, 1 dispenser per 4.67 m ³	100% caged mosquito mortality for 8 weeks, >90% mortality up to 15 weeks	25 bites / hour	70% before 9pm	75% outdoors		6.5 (<1 year)	2.2(<1 year)
[111, 112]	Nigeria	<i>An. gambiae</i> , <i>An. funestus</i>	20% in Wax 1 dispenser per 15 m ³	9-10 weeks	1/49 in treated and control, respectively	Late night peak	Indoors	3.5%	time to first infection 19.6 weeks in control and 25.8 in the treatment	2.5 (>1 year)
[113-116]	Burkina Faso (Upper Volta)	<i>An. gambiae</i> , <i>An. funestus</i>	25% in Wax 1 dispenser per 15 m ³	12 weeks in wet season and 20 weeks in the dry season	50% lower indoor resting density in treated areas but no change in biting pressure	Late night peak	Indoors	Reduced the Sporozoite Rate and correlates well with parasitological outcomes.	Consistent reduction in prevalence of 20 to 50% . Was insufficient to control malaria in the highest transmission months	
[117]	Iran	<i>An. stephensi</i> ,	20% in Wax 1 dispenser per	3-13 weeks depending	Larval density halved, indoor				Malaria declined from 10% slide	

<p><i>An. fluviatilis</i>, <i>An. superpictus</i></p>	<p>7 m³ in ventilated spaces and 1 dispenser per 21 m³ in un-ventilated spaces</p>	<p>on the ventilation</p>	<p>density declined > 5 fold. Population became “younger”. Fewer bites received from <i>An. stephensi</i></p>	<p>positive to 3% slide positive in second season when application occurred before the rains but there was no effect in the first season when application occurred during the rains</p>
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Table 5:2 Overview of insect vectors of disease, their behaviour and means of preventing bites

Sub Saharan Africa: SSA; North Africa and Middle East: NAF &ME; South Central Asia: SCA; Southeast Asia: SEA; Australia: AU; Pacific Islands: PI; North America: NA; Central America: CA; Caribbean: CAR; South America: SA; Europe: EU

Vector	Disease	Location	Time of biting	Indoors/outdoors	Transmission season	Recommendation
1. Anopheles mosquitoes	Malaria	SSA	Dusk to dawn with late night peak	Indoors & Outdoors	All year with peak during and following the rainy season	Avoid mosquito bites especially after sunset by using insect repellents containing DEET or PMD and long clothing
		SA	Dusk to dawn	Mainly outdoors	During and	impregnated with permethrin. Sleep
		CA	with early evening	Indoors & Outdoors	following the	beneath insecticide-impregnated
		SEA	peak		rainy season	bed nets. Sleep in air-
	SCA				conditioned/screened rooms where	
	Lymphatic filariasis	Mainly SSA (75% of cases) some	Dusk to dawn with late night	Indoors & Outdoors		possible and use mosquito coils containing transfluthrin, d-allethrin

SCA, CA, SA,- peak
 mainly in
 residents and long
 term travellers
 >1month

or metofluthrin if possible outdoors
 after dark

2. Aedes
 mosquitoes

Dengue fever
 Rift Valley
 Fever
 Chikungunya

SSA
 SCA
 SEA
 CA
 CAR
 SA
 SSA, ME
 SSA, Naf & ME,

Daytime and early
 evening

Indoors and outdoors

All year round,
 but especially
 following the
 rainy season and
 during epidemics
 During epidemics
 related to very
 high rainfall.

Prevention of mosquito bites during
 daytime using a repellent with
 DEET or PMD is essential during
 epidemics. Use of mosquito coils or
 heated mats indoors and sleeping in
 screened accommodation is advised

		SEA, EU			Chikungunya	Entering EU with climate change.
	Yellow fever	SSA, SA			Can occur year round, but mainly during and following the rainy season and during epidemics	Ensure vaccination for Yellow Fever before travelling to endemic areas.
3. Culex mosquitoes	Japanese encephalitis	SEA		Mainly outdoors	All year round, risk mainly among residents and travellers to rural areas	Ensure vaccination for JE before travelling to endemic areas. Avoid mosquito bites especially after sunset by using insect repellents containing DEET or PMD and long clothing impregnated with
	Lymphatic	SSA, SCA, SEA,	Dusk to dawn	Indoors and outdoors	All year round,	

	filariasis	SA (Haiti, the Dominican Republic, Guyana and Brazil)	with early evening peak		risk mainly among residents and long-term travellers	permethrin. Sleep beneath insecticide-impregnated bednets. Sleep in screened rooms where possible and use mosquito coils containing transfluthrin, d-allethrin or metofluthrin if possible outdoors after dark
	West Nile fever	SSA, NAF & ME, SCA, NA, EU			All year round in tropics, warmer months in northern hemisphere	
4. Sandflies	Leishmaniasis	SSA, SCA, CA, SA 90% of visceral leishmaniasis cases occur in India, Bangladesh, Nepal, Sudan,	Most species are active at dawn and dusk and during the night, but in forests and dark rooms they may also attack in the	Most species feed outdoors but a few feed indoors	All year round	Use long clothing in areas where sandflies are common, as their short mouthparts cannot bite through clothes. Avoid sandfly bites, particularly after sunset, by using insect repellents containing DEET or PMD and by wearing long

Ethiopia and daytime.
 Brazil
 90% of cutaneous
 leishmaniasis
 cases occur in
 Afghanistan,
 Algeria, Iran,
 Saudi Arabia,
 Syria, Brazil,
 Bolivia, Colombia
 & Peru .

clothing impregnated with
 permethrin. Sleep under insecticide-
 impregnated bed nets (small mesh)
 and in screened accommodation if
 possible.

5. Black flies	River blindness	Mainly SSA (West Africa) also CA, SA, mountainous wet areas and southern	Daytime	Outdoors	All times of the year but more common in residents and long term travellers	Avoid areas where black flies are active – near large and fast flowing rivers. Wear long, light coloured clothing treated with permethrin if habitat cannot be avoided
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		Yemen			>3months	
6. Deer flies	Loiasis	SSA (West and Central Africa) in rain-forested areas	Daytime	Outdoors	All times of the year but especially the rainy season and more common in residents and long term travellers	Avoid areas where deer flies are active – near muddy rivers. Deer flies are attracted to wood smoke so avoid campfires. Wear long, clothing. Treat clothing with permethrin if habitat cannot be avoided
7. Biting midges	No disease, but severe nuisance	AU, NA, CA, EU Most northerly temperate regions	Crepuscular during dawn and dusk, but for most species, biting activity peaks in the early evening. Biting in the daytime if	Outdoors	Spring, summer and Autumn when adults are present	Avoid areas where midges are active – breeding grounds are acid, boggy soils or coastal salt marsh. Use repellents of choice. Wear midge hoods. Wear long, light coloured clothing and treat clothing with permethrin if habitat cannot be avoided

conditions are humid, still and cloudy.

8. Tsetse flies	African sleeping sickness	SSA mainly Tanzania, Uganda, Malawi, and Zambia (East African form) Democratic Republic of Congo, Angola, Sudan, Central African Republic, Chad, and northern Uganda (West African	Daytime. East African tsetse prefer wooded thickets and west African tsetse are found in forests and vegetation along streams	Outdoors	All times of year	Avoid wearing dark blue or black clothing. Keep car windows closed when travelling through areas of woodland. Wear long permethrin treated clothing if outdoors in tsetse habitat.
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		form)				
9. Triatomine bugs	Chagas disease	CA and SA, mainly Bolivia	Night	Indoors in rural forested areas, especially in poor housing (mud walls and thatched roofs).	All times of year	Sleep under insecticide-impregnated bed nets. Move the bed away from the wall.
10. Fleas	Plague	SSA, SCA, NA	Day or night	Indoors or outdoors	All times of year	Avoid areas of high rodent density (primary host). Wear a repellent containing DEET and tuck trousers into socks to avoid bites around the ankles. Use an insecticide treated bed net if sleeping in endemic areas
11. Hard Ticks	Tick-borne encephalitis, (TBE)	SCA, EU, SEA (China and Korea)	Day and night	Outdoors	Tropics: any time Temperate: Spring and summer although	Vaccine available in Europe and Canada, but not licensed for use in the USA Avoid areas where ticks are

					season extending due to climate change	abundant in woody and bushy areas with high grass and leaf litter. Walk in the centre of trails.
	Rickettsial diseases including spotted fevers and Q fever	SSA SCA, SEA, NA, EU,				Examine clothes and skin for ticks regularly (at least daily) and remove them with forceps. Wear long clothing and tuck clothing into boots.
	Tularaemia Lyme borreliosis	SSA, NA, EU				Use repellent containing DEET and permethrin on clothing
Soft ticks	Relapsing fever, Borreliosis	SSA, SCA, SEA, NA, EU				
Chigger mites	Scrub typhus	SEA	Any time	Outdoors	All times of the year	As for ticks

Table 5:3 Examples of occupational exposure to disease that can be prevented through the use of repellents

Activity	Region	Disease	Increased risk of disease	Vector	Reference	Prevention strategy	Reference
Illegal Gold Mining	Amazon (Brazil)	Malaria	Prevalence of malaria among individuals involved in gold mining activities (67%) odds ratio 1.92 (1.05-3.50); who came from non-endemic areas (43%) 1.56 (1.06-2.29); and who reported being outside after 5 p.m. (37%) 2.04 (1.06-3.95).	<i>Anopheles darlingi</i>	[118] [119]	Health education, provision of free repellents and or permethrin treated clothing plus long lasting insecticide treated hammock nets to miners. Topical repellents in this region prevent 80% of malaria among users in the Bolivian Amazon odds ratio: 0.20, 95% confidence interval 0.11 to 0.38)	[67] [102]
Open gold mining	Amazon (Bolivar state Venezuela)	Malaria	Malaria was almost absent until the beginning of mining activities in the 1980s. Now between 2001 and 2010 72.3% (22,746 cases) are among men of working age mainly from	<i>An. darlingi</i> and <i>An. marajoara</i>	[120, 121]	Permethrin treated uniforms prevented malaria odds ratio 0.24 (95% CI 0.07-0.87) among soldiers in Colombia – part of the Amazon region	

Overnight forest activities e.g. hunting, travel	Amazon (French Guiana)	Malaria	mining camps 3.3, 95% confidence interval [CI] = [1.1–9.5]	<i>Anopheles darlingi</i>	[122]	
Agricultural expansion into forested areas	Amazon (Brazil)	Malaria	Possibly as a result of their more frequent involvement in forest-related high-risk activities such as clearing land males had a higher malaria incidence (30.7 [95% CI 27.6–34.0] episodes per 100 person-years at risk) than females (21.4 [95% CI 18.7–24.3] episodes per 100 person-years at risk), with a rate ratio of 1.39 (95% CI 1.17–1.64, $P < 0.001$ by Fisher's exact test)	<i>Anopheles darlingi</i>	[123] [124]	
Migrant Forest workers	Mekong (Thailand)	Malaria	Overnight stays in the forest carried a higher risk of malaria infection OR 4.13 (95% CI 1.29-13.13).		[125]	
Overnight forest activities e.g. hunting, travel	Mekong (Lao PDR)	Malaria	Overnight stays in the forest carried a higher risk of malaria infection OR 2.12 (95% CI 1.14—3.95)	<i>An. dirus</i>	[126]	
Collecting food in the forest bamboo, nuts, berries, game animals and	Vietnam	Malaria	Forest work carried a higher risk of malaria infection OR 2.86 [95% CI 1.62- 5.07] in men but not women OR 0.71 (95% CI			Only 2.3% of the population used malaria prevention methods as they cannot afford them. Even after adjusting for the effect of forest work, ethnic group, age, and education, women were still significantly [127]

birds			0.59-0.86)			less at risk of malaria. Compared to men, women usually remain well- covered, particularly when working outside, thus reducing the risk of exposure to mosquito bites.
Rubber tapping	Mekong (Thailand)	Malaria	In an area where LLINs and IRS are applied those earning daily income by performing labor activities mostly in agriculture such as rubber tapping and rubber sheet processing at the smallholdings of rubber plantations were at high risk of malaria odd ratio 2.92 (95% CI 1.14-7.44)	<i>An. dirus</i> , <i>An. maculatus</i> , <i>An. minimus</i> ,	[128]	
Orchards in tropical forested areas	Mekong (Thailand)	Malaria	up to 30% acquired orchards planted on former forested areas	<i>An. dirus</i> , <i>An. minimus</i> ,	[129]	Use of personal protection such as repellents and permethrin treated long clothing if working at dawn or dusk
Organised gold and copper mining	Sumatra (Indonesia)	Malaria	90% of imported malaria between 2009 and 2012 in Sukumbumi health centres (West Java) was among miners from Sumatra who worked night shifts in mines		[130, 131]	Provision of permethrin treated work wear by companies for those on night shift recommended. [101]
Organised open pit gold mining	Iduapriem, Obuasi Ghana; Siguri, Guinea; Sadioloa/	Malaria	2010 Malaria incidence per 100 employees Iduapriem 104.62 Obuasi 19.4 Siguri 22.74	<i>An. gambiae</i> , <i>An. funestus</i> , <i>An. arabiensis</i>	[132] [133]	Use of permethrin treated uniforms did not prevent malaria among soldiers in Thailand although the design of the study may have influenced the results (see section XXX) Permethrin treated work wear for night-shift workers. Insecticide treated clothing prevented malaria by 70% Odds ratio 0.31 (95% CI not reported) in Kenya with <i>An. gambiae</i> , <i>An. funestus</i> , <i>An. arabiensis</i> as the [103]

	Yatela, Mali; and Geita, Tanzania		Sadiola Yatela 9.04 Geita 6.68 despite \$2 million annual investment in control at the sites in LLINs, IRS and health education.		primary vectors	
Military	Netherlands	Lyme disease		<i>Ixodes ricinus</i>	Use of protective clothing and boots reduced the risk of lyme disease in Dutch soldiers based outdoors to that of the control group based indoors	[134]
	New Guinea	Scrub typhus		<i>Trombicula spp</i>	Field tests with dibutyl phthalate applied every 2 weeks to uniforms of Australian soldiers resulted in a 60% and 70% decrease in scrub typhus when it was given to two brigades	[135]
	South Pacific	Scrub typhus			Uniforms were sprayed with dimethyl phthalate or an emulsion formulation of dimethyl phthalate with an untreated control. All of the soldiers then performed combat operations for 7–10 days in areas with scrub typhus transmission. The dimethyl phthalate spray reduced the number of cases by 64% (from 45 cases in the control group to 16 cases in the sprayed group) and the emulsion reduced the number of cases by 94% (to 7 cases).	[136]
	Haiti	Dengue	16 / 241 Italian army troops 30/406 US army troops	<i>Stegomyia (Aedes) aegypti</i>	Skin repellents protective OR 0.16 (95% CI 0.05-0.56), permethrin treated uniform protective OR 0.35 (95% CI 0.11 – 1.17)	[137]
					Although 93 (93.0%) of all febrile patients reported insect bites, only 18 (18.2%) and	[138]

					40 (40.4%) always used topical insect repellent and a bed net, respectively. Few had used permethrin to treat the bed net (30.3%) or uniform (13.1%)		
	Columbia	Cutaneous Leishmaniasis	The greatest outbreak of CL occurred between 2005-2009, with more than 35,000 cases in the military forces, 80% caused by <i>L. braziliensis</i> and 20% caused by <i>L. panamensis</i> .	<i>Lutzomyia. trapidoi</i> , <i>Lu. gomezi</i> , <i>Lu. panamensis</i> , <i>Lu. yuilli</i>	[139]	The soldiers with treated uniforms exposed in an area with infected sand flies for 6.6 weeks had 83% less leishmaniasis (4 cases out of 143 soldiers) compared with soldiers with untreated uniforms (18 cases out of 143 soldiers)	[102]
	Egypt	Sand fly fever		<i>Phlebotomus papitasi</i>		The attack rate (probable immunes were disregarded from the data) among users was 2/77 and among controls was 9/83 = 0.24, which is a 76% reduction	[140]
	Sierra Leone	Malaria	93 cases among deployed UK troops within one month	<i>An. gambiae</i>	[96]	Insecticide treated clothing was protective Odds ratio 0.47 (95% CI 0.20 – 1.05). Use of no personal protection increased the risk of malaria Odds ratio 2.20 (95% CI 0.79-6.17)	[96]
Religious gatherings	Venezuela	Malaria	Evangelic and Catholic Revivalist sects gather outdoors every evening for hymn singing late into the night	<i>An. albitarsis</i> <i>An. oswaldoi</i> <i>An. nunetztovari</i> , <i>An. triannulatus</i>	[141]	Ensure adequate prevention from mosquito bites sing repellents and long clothing	
Missionaries	Haiti	Dengue	After returning from a 1-week missionary trip to Haiti DENV infection was confirmed in seven (25%). None practiced correct	<i>Stegomyia (Aedes) aegypti</i>	[142]		

Workers in the Parks and Forestry Division	North America	Lyme disease	vector bite prevention strategies 6.3% sero-prevalence among forestry workers and the odds of a recalled tick bite were five times higher among outdoor workers	<i>Ixodes dammini</i>		Those who reported that they always used repellent had a 2 - fold lower seropositivity for lyme disease	[143]
	Poland	Lyme disease	In In Poland in 2009, 664 / 10,333 (6.4%) cases were certified as resulting from an occupational exposure among forest workers	<i>Ixodes ricinus</i>	[144]	The use of permethrin treated work wear reduces the probability of tick bites by 93%	[145]
Hiking	North America (Appalachian Trail)	Lyme disease	4% of long distance hikers contracted vector borne disease – principally Lyme disease	Not mentioned but most likely <i>Ixodes dammini</i>	[146]	Subjects wearing treated summer-weight outfits (sneakers, socks, shorts, T-shirt) were 3.36 times (odds ratio 3.36 with a 95% confidence interval (CI) [2.499, 4.526]) less likely to have nymphal I. scapularis attach to their body than subjects wearing untreated clothing. The odds of nymphal attachment, below the waist on the leg where ticks were applied to shoes, were 74 times less (odds ratio 73.60, 95% CI [2.4, 551.45]) for the permethrin-treated group than the untreated group.	[147]
Outdoor recreation – walking, camping, hunting	North America (northwest California)	Lyme disease, human granulocytic ehrlichiosis	Number of nymphs attaching from sitting on logs: 1.44 per hour; gathering wood: 0.42 per hour; sitting against trees: 0.52 per hour; walking: 1.4 per hour; stirring and sitting on litter: 0.32 per hour; sitting on leaf litter: 0.24 per hour.	<i>Ixodes pacificus</i>	[148]		

Table 5:4 Summary of Randomized Controlled Trials with strongest design for evaluation of repellents i.e. those interventions that break man-vector contact: mosquito coils, topical repellents and permethrin treated clothing

SPATIALLY ACTIVE VOLATILE PYRETHROIDS

Trial	Intervention	EPI effect size (odds ratio)	VEC effect size	Primary Vector	Vector feeding behaviour	Compliance	Other points
Syafruddin 2012 [106]	4 x 0.00975 metofluthrin coils per house per night	0.39 (0.24-0.62)	32.9% reduction in mosquito landings by HLC	<i>An. sundaicus</i>	33% of biting before 10pm [149]	Nightly	
Hill 2007 [67]	2 x 0.03 %transfluthrin coils per house per night	0.22 (0.13-0.39)	88% reduction in indoor mosquito densities by CDCLT	<i>An. sinensis</i>	47% of biting before 10pm [66]	>90%	

TOPICAL REPELLENTS

Trial	Intervention	EPI effect size (odds ratio)	VEC effect size	Primary Vector	Vector feeding behaviour	Compliance	Other points
Chen Hussey 2012 [65]	15% DEET lotion in addition to Permanet 2.0 LLINs	0.94 (0.59-1.48)	98.9% protection for 5 hours in field tests	<i>Anopheles dirus</i> , <i>An. minimus</i> and <i>An. maculatus</i>	20 – 50% of biting before 10 pm [150]	About 50%	
Deressa 2010	Buzz off repellent	1.16 (0.75)	> 80% effective	<i>An.</i>	70% before	No measured	Repellent arm had

[151]	plus Permanet LLIN	- 1.80)	against <i>An. gambiae</i> for 8 hours in laboratory tests	<i>arabiensis</i>	10pm [152]		more malaria to begin with. Effect size calculated by study accounting for imbalance was 0.57 (0.35 – 0.94) p=0.028
Hill 2007a [67]	30% PMD lotion in addition to 25mg/m2 deltamethrin impregnated bed net.	0.05 (0.01-0.20)	Repellent provided 97% protection from <i>An. darlingi</i> for 4 hours [73]	<i>An. darlingi</i>	48% of biting before 21.00hrs [71]	>90% (per protocol analysis)	
McGready 2001 [63]	Repellent lotion containing 20% DEET and thanaka (<i>Limonia acidissima</i>)	0.72 (0.50 – 1.05)	Repellent provided 65% reduction in exposure to <i>An. minimus</i> and 85% reduction in exposure to <i>An maculatus</i> [153]	<i>An. minimus</i> and <i>An. maculatus</i>	<i>An. minimus</i> 22% and <i>An. maculatus</i> 62% before bed time	Compliance actively detected at 84.6%	
Onyango 2013 Not published	15% DEET lotion in addition to Olyset LLINs	0.89 (0.69 – 1.13)	Repellent prevented >80% bites from <i>An. arabiensis</i> over 4 hours	<i>An. arabiensis</i>	30% before 10pm	>90% (per protocol analysis) but application not adequately measured	
Rowland 2004 [61]	20% DEET and 0.5% permethrin soap	0.42 (0.25 - 0.69)	<i>An. stephensi</i> and <i>An. culicifacies</i> density–repellent prevented 100% bites over the whole night	100% effective	80% of anophelines biting before midnight	Self-reported compliance >95%	
PERMETHRIN TREATED CLOTHING							
Trial	Intervention	EPI effect	VEC effect size	Primary	Vector feeding	Compliance	Other points

		size (odds ratio)		Vector	behaviour		
Eamsila 1994 [101]	Treated uniform with 2g Permethrin per uniform once every 6 months	0.96 (0.71 – 1.29)	100% effective for 3 months, 84.45% effective up to 6 months	<i>An. dirus</i>	Not measured	100% compliance although it is not known if the uniforms were worn “correctly”	
Kimani 2006 [103]	Clothing treated with 0.37% Permethrin – retreated every three weeks	0.56 (0.36 - 0.86)	41% reduction in blood fed mosquitoes in users’ houses and 41% increase in fed mosquitoes in non-users’ houses	<i>An. arabiensis</i>	Not measured	not mentioned (assume all clothes were treated)	Reported odds of malaria in treatment group is 0.314 p=0.0002
Rowland 1999 [61]	1g/m ² permethrin treated chaddars	0.55(0.38 – 0.78)	Reduced feeding success of <i>An. nigerrimus</i> , <i>An. stephensi</i> and <i>An. subpictus</i> by 0-60%	<i>An stephensi</i>	80% of anophelines biting before midnight	Not measured	
Soto 1995 [102]	Treated uniform with 600-712mg/m ² permethrin	0.24(0.07-0.87)	Not measured	<i>An. darlingi</i>	Not measured	Not measured	

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Chapter 6: General discussion

6.1 Malaria elimination

Long lasting insecticidal nets (LLINs) and Indoor Residual Spraying (IRS) are currently the best methods of malaria control in sub-Saharan Africa [1].

Consequently, their impact on malaria transmission [2, 3] has led to their rapid scale up in the past decade leading to sustainable gains in the control of malaria in most of sub-Saharan Africa [1, 4, 5]. As such, this achievement has encouraged renewed calls for malaria elimination [6]. However, the rapid scale up of these tools has also led to a shift from homogenous to heterogeneous malaria transmission characterized by transmission hotspots in sub-Sahara Africa [7, 8]. It follows therefore that; further scale up of current malaria control tools will not have any additional impact on malaria transmission in these residual transmission foci [9, 10].

Accordingly, new tools and strategies will be needed to achieve this goal [11]. While development of parasite resistance is a major concern in South America [12], development of outdoor transmission presents a real challenge in sub-Saharan Africa [5], and is the focus of this thesis. LLINs and IRS cannot tackle transmission outdoors as they are predominantly intra-domiciliary control tools. A potential strategy would be the use of repellents when people are outdoors, either on their person (topical repellents) or in an area occupied by people outdoors (spatial repellents). Repellents can be used outdoors during the day and in the early evenings before users go into insecticide-sprayed households or under their LLINs.

6.2 Evaluating the efficacy of 15% DEET topical repellents against mosquitoes

Efficacy of the 15% DEET topical repellent used for these experiments was established in the semi-field and field setting before it was implemented in the community. The topical repellent, 15% DEET, provided 85 % and 82 % protection against wild mosquitoes and laboratory reared *An. arabiensis* in the field and semi-field setting for over four hours respectively. This finding is similar to several studies that have evaluated efficacy of DEET against mosquitoes in the field [13-22].

This study is the first to evaluate the efficacy of repellents in a SFS and compare the findings to field evaluations and demonstrated that repellents provided greater protection in the field compared to the SFS even though there was higher biting density in the SFS [23]. This demonstrates that repellents that provide adequate protection in the SFS are more than likely to give greater protection in the field.

However, it's prudent to note that these experiments compared the efficacy of 15% DEET topical repellent against a single species of mosquito and findings should not be generalized for all mosquito species. It is important to assess repellents in the SFS against all available major vector species for the study area (e.g. for Tanzania both *An. gambiae* and *An. arabiensis* since *An. funestus* is not easy to colonize) and nuisance species such as *Cx. quinquefasciatus* if possible to ensure that the repellents work well to stop all bites and encourage compliance. Another interesting finding from this study was the effect of formulated against unformulated repellents. It has been often assumed that formulated repellents (polymer formulations and microencapsulation) last longer when applied on the skin, because the polymers and microencapsulation reduce volatility and skin penetration [24]. However, this study demonstrated that unformulated 15% DEET diluted in ethanol provided similar

protection against mosquito bites compared to formulated 15% DEET lotion in the field similar to other studies evaluating efficacy of repellents [25, 26]. These findings suggest that the concentration of active ingredient (A.I.) might be more important in determining the efficacy of repellents relative to the repellent formulation.

It is also necessary to establish the correct effective doses required to provide effective protection for different time periods, against different vector species, to avoid exposing individuals to higher treatment doses than necessary.

It would also be prudent that repellent evaluations are carried out throughout the night so that its possible to model half-life and effective doses of repellents for different time periods [13].

6.3 Mode of action of DEET

Despite its long-term and extensive use, the exact *modus operandi* of DEET is yet to be established, with a few existing theories attempting to explain its mode of action.

The first theory suggests that DEET acts by inhibiting the response to a usually attractive chemical signal. It is suggested that, DEET interferes with the electrophysiological response of receptors for carbon dioxide, lactic acid and 1-octen-3-ol, all of which are attractive mosquito odorants [27], so that the mosquito is not able to recognize and interpret these stimuli [28, 29]. This hypothesis has however been refuted by experiments showing that mosquitoes still avoid DEET in the absence of these odorants [30]. DEET does not interfere with the electrophysiological response of ORNs to 1-octen-3-ol, but rather reduces the amount of attractant reaching the receptor neurons, hence a false-positive inhibition of 1-octen-3-ol neurons [30].

The second theory suggests that mosquitoes have olfactory receptor neurons that are specific to DEET, and as a result they detect and avoid DEET. This proposed mode of

action is supported by the fact that mosquitoes were observed to avoid DEET in the presence of physical stimuli only and the identification of DEET sensitive ORNs in the antennae of *Culex quinquefasciatus* [30]. The proponents of this theory also demonstrated that DEET might have a fixative effect when applied on the human skin, preventing the release of physiological compounds that are required for host location, e.g. lactic acid [30].

The last mode of action proposed was that DEET acts by activating gustatory receptor neurons (GRNs) that mediate aversive behaviour of the mosquitoes. It suggests that GRNs and not ORNs are used to detect DEET and cause the mosquito to move away from the source of DEET. This theory is supported by experiments that show mosquitoes in which ORNs that mediate DEET response, OR83b, have been ablated still avoid DEET. However this assessment is biased as mosquitoes in which GRNs that mediate DEET response, Gr33a should also have been inactivated and these mutant insects tested against DEET to determine if these mosquitoes were able to detect DEET.

From the evidence presented above it is likely that DEET does not interfere with reception of signals, but instead there is a DEET-specific ORN in the mosquito maxillary pulp that mediates aversion to DEET. It is also likely that DEET is detected by mosquitoes in the vapour-phase through olfactory chemo-receptors, ORNs, as well as contact chemo reception through GRNs [31].

In the current study testing the efficacy of DEET [15], it was observed that DEET reduced bites from all mosquito species that were present in the field. Although tests were not carried out to assess the impact of 15% DEET against each mosquito species, results indicated that each mosquito species detected and avoided DEET.

Further, one volunteer received twice as many mosquito bites as the other volunteers

[15]. An individual who is more attractive to mosquitoes is expected to produce more lactic acid, CO₂ or 1-octen-3-ol than other volunteers. It is therefore logical to expect that if DEET interferes with the electrophysiological response to these odorants, then the individual would receive an equal number of bites as the rest of the volunteers when using DEET, as the effect of these odorants would have been neutralized. However this was not the case as this individual was found to receive more mosquito bites whether they were wearing the DEET repellent or not. This finding further reinforces the fact that DEET does not inhibit the attraction of these odorants. From the above theories, it is still not possible to determine the mode of action of DEET further research is recommended in this area.

6.4 Characteristics of a good repellent

Ideally a good repellent should be broad-spectrum; have an effect on multiple species of biting arthropods [32]. As observed from the efficacy study above (Chapter 3), 15% DEET repellent used in this trial protected against a wide range of mosquito species including, *Anophelines*, *Culicines*, *Mansonia* and *Coquillettidia* species [15]. A good repellent should reduce biting by at least 80 % for 6-8 hours [33]. This characteristic would make this repellent ideal for integrated vector control strategy in this community, as it would offer the community protection from dawn up to the time bed nets are employed.

It should also be non-irritating to the skin or mucous membranes after application and should have a pleasant odour or at least be odourless and greaseless, making it cosmetically appealing so as to encourage uptake of repellents. A good repellent should also be inert to commonly used plastics, i.e. it should not damage these surfaces. Lastly a good repellent should be easily available and economically accessible even to the poorest individuals in the society so as to improve its use.

To date, there is no repellent that meets all of the above criteria [34, 35]. Currently, the ‘gold standard’ repellent is *N, N-diethyl-m-toluamide* (DEET). Although DEET has been shown to be effective against a wide range of arthropod vectors [36] and to have adequate longevity [25], it is often reported to have an unpleasant odour, irritate the mucous membrane and damage plastic, a characteristic that often makes this product be perceived as unsuitable for topical application. However, DEET has been evaluated and recommended by the EPA as safe for human use [14] and is currently the longest lasting and most widely used repellent available [37].

6.5 Evaluating the effect of 15% DEET topical repellent against malaria transmission

The cluster-randomized trial of topical repellent did not demonstrate any significant impact on malaria incidence.

These findings demonstrate that 15% DEET topical repellent may not have any effect on malaria transmission in the early evening in rural Tanzania, and are consistent with findings of a study carried out in Lao-PDR [38]. Both the current study [39], and the Lao-PDR study used 15% DEET repellent, while other topical repellent studies which have shown DEET to have an impact on malaria transmission used 20% DEET, [40, 41]. It can be argued that concentration of 15% DEET repellent was too small to have an epidemiological impact on malaria. However, the Lao-PDR study tested three different concentrations of repellents where 15% and 20% DEET gave a similar amount of protection, 98% against mosquitoes. Therefore the concentration of repellents used is unlikely to have resulted in the observation of no effect between the treatment arms in both the current and Lao-PDR studies. However, even though the above studies did not demonstrate a significant result, there are a few randomized

controlled studies where topical repellents have had varying outcomes on malaria transmission [41-44].

As all the above studies demonstrated varying effects of topical repellents against malaria transmission, the lack of effect observed in some of the above as well as the current and Lao-PDR studies were likely a result of flaws in the study design.

6.6 Situations where repellents can be used to complement current control tools

Albeit effective, LLINs and IRS have had an impact on malaria epidemiology in sub-Saharan Africa, so that increased use of these tools has led to a shift to early evening biting, outdoor biting, zoophagic and outdoor resting vectors [46]. As a result, new tools will be needed to tackle transmission at these settings, and topical repellents may provide a practical solution in such settings.

Topical repellents can be used in areas where there is early evening and outdoor biting [41, 47-52] of malaria vectors.

There have been several reports of increased exophagy as a result of increased use of LLINs [50, 55-58]. All these reports illustrate the potential of these outdoor vectors mediating residual transmission despite extensive use of current tools, case in point being Benin [58] [58], where universal coverage of LLINs was achieved and yet there was still malaria transmission. Topical repellents can be applied in these situations so that individuals are protected in the early evening before they can employ LLINs.

Different occupations such as plantation work and forest activities have been shown to expose the community to outdoor mosquito bites at times when LLINs and IRS could not be used [39, 59-65]. Long lasting and sweat resistant repellents can be employed at these times to protect these individuals by preventing human-vector contact.

Extensive use of LLINs and IRS has led to wide spread insecticide resistance [66-68]. Development of resistance is likely to have a great impact on the gains that have already been made in malaria control, such as was seen in the resurgence of malaria Kwa-Zulu Natal project that used deltamethrin on pyrethroid resistant *An. funestus*. [69]. Topical repellents can be used in areas where there is development of resistance, while efforts are being made to develop new insecticides. This strategy is likely to slow development of resistance, as repellents have low selection pressure because they do not kill vectors but only move them away from the hosts [70]. Caution should however be taken when implementing topical repellent coverage. In areas incomplete coverage, diversion has been observed from repellent users to non-users [22]. It is therefore advisable that when topical repellents are being used, compliance and uptake are encouraged to avoid exposing non-users to more potential malaria than normal.

6.7 Feasibility of topical repellents as a malaria intervention tool

Mosquito repellents are used predominantly in developed countries [71], where malaria transmission has been eliminated, usually in cases of disease outbreaks [37], or by tourists travelling to malaria-endemic regions [72]. However, in sub-Saharan Africa, where malaria until recently has been endemic, repellents would likely not have any impact on transmission, because of their poor longevity and need for frequent reapplication, leading to poor uptake and hence effectiveness. As a result, other malaria control tools like LLINs and IRS, which have better longevity (up to 5 years) and compliance (as it requires to be hung only once by the user) and therefore better impact on malaria transmission were advocated, socially marketed and implemented at the expense of repellents. Therefore, poor use of repellents is due to lack of awareness of this mosquito control tool [23].

However, in the current study, after community sensitization, repellents were readily used in this community in rural Tanzania, with self-reported compliance at ~ 80% through out the study period, similar to other studies that reported better uptake and acceptability of interventions as a result of increased community awareness [73-76], further supporting the need for community sensitization before implementation of interventions.

Bed nets were the preferred mosquito control tool used in the study area, mainly because of its reported cost effectiveness. The community found it cheaper to use a bed net that lasted ~ 5 years before requiring replacement, than repellents that would require frequent replacement, mostly monthly, and therefore cost more in the long run, demonstrating that in a resource limited community, cost influences the choice of an intervention [74, 77, 78], and that cheaper cost-effective repellents are preferred to more costly interventions.

Ease of use was another key reason for bed net preference. Topical repellents require the user to remember daily applications. The community found this attribute cumbersome and preferred to use LLINs, which needed to be hung only once and used for up to 5 years. This finding demonstrates that interventions being advocated should be tailored to address user preferences. Apart from user friendliness, an intervention needs to be effective. In this study, interventions that were easy to use but not effective, like the sisal strip impregnated with transfluthrin, had poor uptake. This finding indicates that interventions need to be user friendly as well as effective, similar to findings of a mathematical model, which established that the effectiveness of any repellent was dependent on its efficacy and compliance [79],(Moore and Briet, in preparation).

The main reason for non-compliance to repellent use was forgetfulness. It is therefore necessary to explore the different formats under which repellents can be presented to promote uptake. When explored in this study, it was observed that insecticide treated clothing [80, 81] would yield better compliance. Additionally, presentation of repellents in formats that do not require the user to remember daily compliance such as spatial repellents need to be explored [70].

However it should be noted that, despite all the above shortcomings, topical repellents were the preferred means of protection in the early evening before users retired under their LLINs. This finding demonstrates that with improved advocacy, cost, ease of use and better formats, repellents can be a feasible malaria control tool in this community in rural Tanzania in the early evening.

6.8 Impact of repellents against malaria transmission

Several randomised controlled trials have indicated an impact of topical repellents on malaria transmission [38, 40, 42, 44, 45, 82], when used in conjunction with LLINs compared to singular use of LLINs. Mosquito control is usually aimed at preventing the human-vector contact and thereby reducing the vectorial capacity of the mosquito. Even though topical repellents do not reduce vector density, by killing the mosquitoes, it reduces the biting rate of the mosquito on humans by preventing the human vector contact. A reduction in the man-biting rate has a two fold impact of the vectorial capacity compared to reduction in vector density [83]. Therefore, by lowering the biting rate, topical repellents will have a two-fold decrease on the vectorial capacity. Topical repellents will also influence the survival rate of the mosquito, as preventing human-vector contact impacts mosquito longevity [84]. Therefore while LLINs lower the vectorial capacity of the vectors throughout the night, using repellents in the early evening will impact on the vectorial capacity in the

early evening (through reduction of man biting rate and survival rates) and therefore reduce transmission further than in areas where repellents are not used in the early evening.

Mathematical models have shown that combining DEET and LLINs increased protection against mosquito bites [85], further supporting the theory that combining these two strategies have greater impact on malaria transmission.

This finding is further supported by the randomized repellent studies, which have demonstrated an epidemiological impact of repellents against malaria transmission [40-42, 44, 82].

6.9 Conclusion and future work

Repellents that protect against mosquito bites in the SFS are likely to provide greater protection in the field and it is recommended that evaluations of topical repellent be conducted in the SFS to avoid exposing volunteers to potential malaria infection.

The cluster-randomized controlled trial of 15% DEET topical repellent against malaria in rural Tanzania found no evidence of an effect on malaria transmission in the early evening. The lack of treatment effect is likely a result of poor study design and implementation and better-designed studies should be conducted to evaluate the impact of topical repellents on disease.

Community sensitization and education are required to encourage uptake of interventions introduced in the community. These interventions must however be cost-effective and user-friendly, tailored to meet the community needs and preferences. An additional and essential caveat is that interventions that require a change in human behaviour, like frequent application, have initial poor uptake hence effectiveness. Use of tools like insecticide impregnated clothing, which are likely to have better uptake need to be explored in this community.

Methodologies assessing the impact of similar interventions should be standardized to make the findings easy to assimilate and assess in systematic reviews and meta-analyses so that the overall effect, (protective efficacy – PE), of such interventions can be easily established.

Malaria transmission in sub-Saharan Africa is changing and there is need to develop tools that will tackle malaria at places and times beyond the control of current tools. Outdoor malaria transmission is quickly developing into a focus of malaria transmission and needs to be tackled to sustain control and possibly achieve elimination. Based on the findings of this work, 15% DEET repellent has been shown to protect against outdoor and early evening biting mosquitoes but not malaria transmission. Wide scale use of repellents cannot therefore be recommended based on the findings of this work alone without further research. Alternatively, the impact of other modes of repellent delivery formats like spatial repellents and insecticide treated clothing also need to be explored.

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Chapter 7: Study Protocol: Evaluation of the efficacy Permethrin impregnated clothing (PIC) against malaria transmission outdoors in rural Tanzania; A cluster-randomised, placebo-controlled trial design.

7.1 List of abbreviations

ACT	Artemisinin Combination Therapy
AI	Active Ingredient
ATP	According to Protocol
CHW	Community Health Worker
CRF	Case Report Form
DED	District Executive Officer
DHMT	District Health Management Team
DMO	District Medical Officer
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Environmental Protection Agency
GCP	Good Clinical Practice
HIN	Household Identification Number
HLC	Human Landing Catch
IHI	Ifakara Health Institute
IRB	Institutional Review Board
IRS	Indoor Residual Spraying
ITT	Intention to Treat
LLIN	Long lasting insecticidal net
LTFU	Loss to Follow up
PCR	Polymerase Chain Reaction
PC	Project Co-ordinator
PE	Protective Efficacy
PI	Principle Investigator
PIC	Permethrin-impregnated clothing
PL	Project Leader
RDT	Rapid diagnostic Test

SAE / AE	Serious Adverse Events / Adverse Events
SFS	Semi Field System
SOP	Standard Operating Procedure
SR	Spatial Repellent
TCU	Ten Cell Unit
TCUL	Ten Cell Unit Leader
UIC	Unique Identification Code
UIN	Unique Identification Number
VEO	Village Executive Officer
VHCW	Village Health Care Worker

7.2 Background

Extensive injection of financial and political resources in the last two decades into malaria control programmes [1], has led to scale up of current control tools, like long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) [1]. Other tools being widely used in malaria control include rapid diagnostic tests kits (RDTs), used for prompt diagnosis of malaria cases [2]. Rapid diagnosis has led to timely treatment of malaria cases and reduction of the number of cases treated presumptively, hence lowering the risk of drug resistance development. Treatment of malaria with combination therapies (Artemisinin Combination Therapies – ACT), is also being expansively employed in sub-Saharan Africa [3, 4]. Use of combination therapies also lowers the risk of drug resistance development, which is often seen in areas where mono-therapies are used [3, 5]. As a result of large scale use of these control tools, there has been substantial decline in malaria morbidity and mortality in sub-Saharan Africa and other WHO regions [1] and consequently renewed calls for malaria elimination [6].

Accordingly, extensive employment of these tools is likely to bring about rapid change in malaria epidemiology in sub-Saharan Africa [7]. First, wide spread use of LLINs and IRS, is likely to have a major impact on the malaria vector by selecting for vectors that bite outdoors and in the early evening before they are employed [8, 9]. Therefore, despite extensive employment and significant impact on malaria transmission [10, 11], these tools will not be sufficient for malaria elimination. LLINs and IRS predominantly protect against intra-domiciliary malaria vectors that bite at night indoors, and rest indoors. Therefore, vectors that bite during the early evening or morning as well as during the day, when people are active, and not under bed nets and those that rest outdoors, will still maintain malaria transmission, albeit at low

levels, even with complete LLIN and IRS coverage [12-14]. Such a scenario may result in malaria epidemic outbreaks and resurgence because of diminished background immunity of the population as a result of reduced exposure to infection that results from successful malaria control [13].

Secondly, urbanization, rapidly taking place in Africa, has led to electrification of rural communities, and as a result the community members stay awake for longer than they usually would, resulting in extended exposure to mosquito bites in the early evening [15].

Development of resistance of malaria parasite to currently used antimalarials has been detected in South Asia [16], and threatens to spread to other malaria endemic areas if not controlled at the initial stages [1, 17].

Therefore, even though LLINs and IRS have had a far-reaching impact on malaria control, they are unlikely to eliminate malaria [10-12, 18] because the above factors are likely to attenuate their effectiveness. As a result, there is need to develop novel tools that can protect individuals and communities outside of sleeping hours and that can complement current control methods at times and places when their effectiveness is diminished.

Use of topical repellents has demonstrated varying outcomes in complementing LLINs against early evening malaria transmission:

In South America, a double blind, randomized controlled trial assessing the efficacy of 30% para-methane-3-diol (PMD) repellent, demonstrated a significant reduction in *Plasmodium vivax* malaria transmission, while there was no effect on *Plasmodium falciparum* malaria. The major reason given for no observation of an intervention effect on *P. falciparum* malaria was lack of statistical power as *P. falciparum* malaria cases were too few to observe a treatment effect. This was because the study took

place for only four months when *P. falciparum* malaria transmission was low. A study conducted for longer would have likely demonstrated an effect against *P. falciparum* malaria [19].

In another household-randomized, placebo controlled trial in a refugee camp in Pakistan, a topical repellent soap containing 20% DEET and 5% permethrin was evaluated for efficacy against *P. falciparum* and *P. vivax* malaria transmission. The DEET and permethrin containing soap demonstrated a 56% reduction in malaria transmission in the intervention group compared to the control group. There was however no impact on *P. vivax* malaria transmission as the study took place for only six months when *P. vivax* transmission was too low to demonstrate a statistically significant treatment effect. Similar to the above trial, a treatment effect would likely have been observed if the study was conducted through both the *P. falciparum* and *P. vivax* transmission seasons. This study also used passive case detection which might have led to loss of malaria cases not presenting at the hospital, consequently biasing the treatment effect downwards [20].

In a case-control study in South Asia, carried out in Afghanistan villages, through social marketing of Mosbar, a repellent soap containing 20% DEET and 5% permethrin demonstrated a 69% reduction in the odds of contracting malaria in the intervention group. However, this study was conducted as a health-facility based study, which is likely to have biased the outcome, and therefore the findings could not be generalized to the whole population [21].

In another double blinded randomized controlled trial in Southeast Asia, Thailand, use of DEET and *thanaka* did not demonstrate any impact on the transmission of *P. falciparum* or *P. vivax* malaria [22]. The lack of effect was likely a result of effective malaria management in the study area and an effect may have been observed if the

study took place in an area where no other malaria intervention was being implemented.

In sub-Saharan Africa, a cluster randomized controlled trial in Ethiopia using of Buzz Off repellent with LLINs demonstrated a 34% reduction in malaria transmission. However, the amount of active ingredient used in the repellent is not given. Also the baseline malaria prevalence was higher in the intervention compared to the control group. The investigators also did not follow the analysis plan when analyzing the data. All these factors are likely to have biased the findings of the study and outline important details that should be taken into account when designing and implementing studies using similar interventions [23].

In Ghana, efficacy of NO-MAS topical repellent, evaluated in a community trial in two villages demonstrated a 19.2% reduction in malaria in the intervention group compared to a 6.5% reduction in the control group. However poor reporting of allocation concealment, baseline characteristic and blinding of participants will likely have biased the implementation and findings of this study [24].

A recently conducted meta-analysis of randomized and non-randomized topical repellent trials suggested that use of topical repellents is ineffective against preventing malaria transmission. However, because of the heterogeneity of the ecological and entomological indices in the different study areas where these studies were conducted, it is prudent that a standardized methodology of implementing such studies be established so that it is easier to synthesize results of different studies and establish whether this intervention has any protective efficacy (PE) against malaria transmission [25].

One of the major drawbacks of using topical repellents is ensuring compliance.

Topical repellents require daily application, and at times, more than one application in

a day, which makes its use difficult, as users have to remember to apply the repellent frequently [26]. Secondly, topical repellents require frequent replacement as they are bound to run out at a faster rate than other control tools [27]. This attribute makes them more expensive compared to other control tools like LLINs, which can be bought once and used up to 5 years, or IRS, which lasts up to 6 months after one round of spraying [28]. These aspects of topical repellent makes its compliance challenging [27], which in turn lowers its effectiveness, a product of efficacy and uptake [29] (Moore and Briet et al, in preparation).

Passively emanating spatial repellents is another tool that can be used to complement LLINs and IRS. Spatial repellents are likely to have better compliance compared to topical repellents, as they do not require daily applications. They only need to be put up once, like LLINs, and used for the period that the active ingredient (A.I) is effective. They are also more cost-effective than topical repellents as they can protect more than one individual in a designated area at any given time [30, 31]. However SR repellents are still not as cost effective as LLINs and its implementation may not be sustainable in resource-limited communities, as they will need to be replaced more frequently than LLINs, and as a result, making it an expensive endeavor to both the users and to donor organizations and local governments in form of subsidies to the population. Also, SR products may divert mosquitoes, from users to non-users in incomplete coverage scenarios [32], thereby requiring complete coverage, an achievement which is unrealistic in intervention implementation programmes [33]. There have been two randomized controlled trials to assess the efficacy of spatial repellents against malaria.

In Yunnan province in China, a single-blind randomized controlled trial assessing the efficacy of 0.03% transfluthrin coils against malaria prevalence, demonstrated a 75% reduction between the intervention group and the control group where no intervention was employed. However, when use of coils was assessed against use of coils and LLINs, then there was no significant difference between these two groups, even though, the LLIN plus coil group demonstrated greater protection from malaria than the coils alone group [34].

Another randomized, double-blinded, placebo controlled trial to determine the effect of metofluthrin coils on malaria incidence was conducted in Sumba, Indonesia.

Metofluthrin was found to be 52% protective against contracting malaria. However, when adjusted to account for clustering, no difference was observed between the intervention and control groups [35].

Although these trials demonstrated a protective trend of coils against malaria, they did not conclusively demonstrate the effect spatial repellents, and both recommend that larger, statistically powered trials be conducted to establish the effect of spatial repellents on malaria transmission.

Therefore to conclusively establish the effect of spatial repellents on malaria transmission, a multi centre, cluster randomized, double-blinded, placebo controlled trial using passive transfluthrin emanators is currently being conducted in Kenya, Tanzania, Zambia and Indonesia whose results are expected in 2016.

Another potential strategy is the use of permethrin-impregnated clothing (PIC) to complement LLINs and IRS. Permethrin, the main insecticide recommended for impregnating clothing, was approved for this use in 1990 by the Environmental Protection Agency (EPA), [36], and has been evaluated for safety from 1970-1990.

The evaluations done during this period include: dermal and oral toxicity, mutagenicity and teratology, skin and eye irritation and determination of efficacy of impregnation with different concentrations. Further tests include evaluation of metabolites in urine after using permethrin-impregnated clothing and migration of permethrin from cloth to skin, fabric-skin contact and finally the effect of washing on permethrin retention and efficacy [37]. Permethrin has demonstrated low mammalian toxicity, hence recommended as safe for human use [37]. Permethrin is also considered safe for children as it's widely used for treating head lice and scabies in children and neonates [38]. It is also a broad-spectrum insecticide that knocks down as well as repels arthropods [39, 40]. Compared to other pyrethroids, permethrin is very stable when exposed to U.V. light, making it a suitable insecticide for impregnation of clothing, as users will be exposed to sunlight when using this intervention. It is also non-staining on clothing and has a residual effect ranging from 6 weeks to one year depending on the impregnation method used [41]. It is usually used with a binding agent during impregnation that enables the insecticide to bind to the clothing even after several washes.

Several studies have been conducted to evaluate the efficacy of PIC against arthropod bites as well as disease transmission with varying outcomes:

In Harford County, US, the efficacy of permethrin-impregnated, permethrin-sprayed and DEET-treated battle dress uniforms (BDU) was evaluated against biting ticks in the field and compared to an untreated control. The permethrin-impregnated, sprayed and DEET treated BDU reduced biting ticks by 97%, 98% and 60% respectively [42]. Field trials in Oklahoma District, US, evaluating the effectiveness of 0.5% permethrin single spray applications on cotton clothing for 15, 30 and 60 seconds provided 100%

protection for 1 hour each day, when tested for three consecutive days against tick bites [43].

In a laboratory experiment in the US, evaluating the efficacy of five chemical repellents and a permethrin-impregnated fabric, permethrin was found to provide complete protection against *Aedes albopictus* and *Aedes aegypti* after 5 washings, while the other chemical repellents provided protection that lasted for only up to 10 hours [44].

Field evaluations of permethrin-treated clothing and extended duration DEET against local mosquito vectors in Islamabad, Pakistan, demonstrated a 57% protection against mosquito bites for permethrin-treated clothing and 89% protection for the extended formulation DEET. However, the combination of permethrin-treated clothing and DEET provided 100% protection against mosquito bites [45].

In Zambia, permethrin-impregnated clothing and three DEET topical repellents evaluated against a natural population of tsetse flies demonstrated 34% and 76-87% protection respectively against tsetse fly bites. In addition, combining permethrin-impregnated clothing and DEET repellent provided 91% protection against tsetse fly bites [46].

In Australia, use of DEET with permethrin-treated clothing provided greater protection against mosquito bites compared to use of either DEET or permethrin-treated clothing alone. However permethrin treated clothing and DEET both significantly reduced the number of mosquito bites compared to the control [47].

While field and laboratory evaluations demonstrate that permethrin-treated clothing is effective against mosquito and other arthropod bites, epidemiological studies have been more variable.

In a randomized controlled trial in an Afghan refugee camp, in Pakistan, permethrin-impregnated *chaddars* reduced the odds of *P. falciparum* and *P. vivax* malaria by 64% and 38% respectively for individuals below 20 years, but had no effect on individuals above 20 years of age. The study was conducted for 5 months at the beginning of the *P. falciparum* transmission season, hence the observed low efficacy of the intervention against *P. vivax* malaria. This study also used passive case detection of malaria cases and it is likely that cases that did not present at the health facility were missed, thereby underestimating the intervention effect. Compliance was also established by self-reporting. A study conducted for longer, with better malaria data capture methods and establishment of compliance would have likely demonstrated a greater intervention effect [48].

In another randomized controlled trial, in Afghanistan, insecticide treated *chaddars* reduced the efficacy of cutaneous leishmaniasis by 65%, and had similar efficacy to that of insecticide treated bed net use. This study was well powered as it had sufficient number of participants to demonstrate a treatment effect and was conducted for 15 months, which captured the heterogeneity of malaria transmission in different seasons. However, compliance to permethrin-impregnated *chaddars* could not be established and therefore the treatment effect observed could not be conclusively linked to the use of this intervention [49].

A double blinded, placebo controlled trial, evaluating the effect of permethrin-impregnated uniforms worn by the Royal Thai army, in the Thai-Cambodia border did not demonstrate any effect of permethrin-impregnated uniforms on malaria incidence. Compliance to this intervention could not be fully established and it is likely that the lack of treatment effect was as a result of non-compliant participants biasing the intervention effect towards the null hypothesis. Randomization of the study

participants might also have been biased leading to the observation of no effect of permethrin-treated uniforms [50].

In Colombia, a double blinded, placebo controlled trial among Colombian soldiers using permethrin-impregnated uniforms demonstrated a 75% and 79% protection against cutaneous leishmaniasis and malaria transmission respectively. Active case detection was employed in this study and it is unlikely that any cases were missed. However, compliance could not be fully established, and therefore the treatment effect observed might have been confounded. Also, the sample size used and malaria and CL cases reported between the two groups were too small to yield credible findings [51].

In a community trial conducted among Somali refugees, in Dadaab refugee camp, Kenya, use of permethrin treated clothing reduced the odds of contracting malaria by 70%. Similar to the above study the sample size used in this study was small and reporting of treatment effect was unclear [52].

In another Kenyan community trial, use of permethrin-impregnated clothing was associated with 90% protection against malaria transmission in participants above 6 years. There was however no intervention benefit for children less than 5 years. The sample size was calculated from health facility reports, which led to underestimation of sample size needed to observe a treatment effect. Further, there was incomplete intervention coverage for children under five years of age. These two factors, a small sample size and incomplete coverage might have biased the study and masked the effect of the intervention in children under five [53].

As malaria control moves from sustained control to elimination, there is need to develop tools that attack transmission at times when the effectiveness of current

control tools are diminished. Topical and spatial repellents present potential options for this strategy. However, both these tools present challenges in their implementation. Topical repellents require daily and optimum applications by the user, hence resulting in difficulty in uptake and use. As effectiveness of interventions is largely dependent on uptake, poor uptake of topical repellent will likely diminish its effectiveness [29], making it unsuitable for large-scale implementation.

While spatial repellents may overcome the challenges of daily application required for topical repellent implementation, and therefore resulting in better uptake, it requires complete coverage so that vectors are not diverted from users to non-users [32]. This presents a challenge, as complete intervention coverage is not feasible under real life conditions [33]. As a result, use of spatial repellents may expose non-users to more malaria than normal.

On the other hand, all communities in the world already use some form of clothing. Therefore deployment of insecticide through the use of clothing that are already in use in the community is likely to have better uptake and higher coverage compared to topical and spatial repellents. As clothing is used throughout the day, it is expected that permethrin impregnated clothing will provide protection throughout the day and LLINs can be used to provide protection overnight. This way there will be an all-round protection of PIC users from arthropod bites. In doing so, PIC cannot only be used to protect not only against malaria, but also against other arthropod-borne diseases like Dengue, which is emerging to be a disease of public health importance in Tanzania [54, 55]. As a result of arthropods having no access to human hosts, it is expected that transmission of arthropod-borne diseases, in this case malaria, will significantly decline. Another advantage of PIC is that it does not interfere with the daily activities of the users, which is also likely to encourage uptake.

A study conducted recently in rural Tanzania, assessing the feasibility of various intervention formats established that the community would readily use permethrin-treated clothing [27].

Therefore, taking into account the potential benefits of PIC over topical and spatial repellents, and the shortcomings in the design and/or implementation of permethrin-impregnated clothing studies outlined above, this study was designed to evaluate the benefits of using permethrin impregnated clothing (PIC) and LLINs in preventing transmission of *Plasmodium falciparum* malaria in a community in rural Tanzania.

7.3 Rationale

Current control tools, LLINs and IRS, will not be able to eliminate malaria mainly because they do not protect against populations of malaria vectors that bite before they are deployed. There is therefore need to develop novel tools to complement these current control strategies against mosquito populations biting at times and places when their effectiveness has been diminished if the goal of elimination is to be achieved. Permethrin-impregnated clothing require minimum compliance as clothes are already used worldwide and they can be used throughout the day compared to other complimentary tools like topical and spatial repellents, which are deployed mainly in the early evening or when individual are at designated locations where these tools are present. This integrated use of PIC and LLINs therefore presents a potential strategy for protecting against residual malaria transmission mediated by mosquito populations against which the effect of LLINs and IRS are attenuated. We propose to carry out a cluster-randomized, double blinded, placebo controlled intervention trial to evaluate whether there is any benefit of using PIC and LLINs combined compared to use of LLINs and a placebo against malaria transmission.

7.4 Objectives

7.4.1 Clinical objectives

7.4.1.2 Primary objectives

- To assess and quantify the protective efficacy (PE) of permethrin impregnated clothing (PIC) in reducing the incidence of *Plasmodium falciparum* malaria infection in a rural community in Tanzania as measured by active case detection using PCR diagnosis

7.4.1.3 Secondary objectives

- To assess and quantify the protective efficacy (PE) of permethrin impregnated clothing (PIC) in reducing the incidence of malaria infection in a rural community in Tanzania, as measured by rapid diagnostic tests (RDT).

7.4.2 Entomological objectives

- To quantify a reduction in vector biting densities, sporozoite and parity rates of malaria vectors in the intervention group relative to the control group.

7.5 Study endpoints

7.5.1 Epidemiological endpoints

Primary: The incidence density of malaria infections among study participants over the follow-up period as detected by PCR, to inform the reduction of malaria incidence between intervention and control group based on an expected minimum effect size of 30% using the formula:

$PE = [(I_p - I_a)/I_p] * 100\%$; where;

I_p is the incidence density in the placebo (control) group

I_a is the incidence density in the intervention (active) group.

Secondary:

Determine the PE of PIC against malaria infections as detected using RDT by comparing the incidence densities of malaria between the intervention and control group.

Determine the PE of PIC against malaria related anaemia as measured by HemoCue, by comparing the incidence densities of anaemia between the intervention and control group.

7.5.2 Entomological endpoints

Primary: Proportion of adult malaria vector biting densities assessed via human-landing catch (HLC) from households in the intervention and control group over the follow-up period.

Secondary: Proportion of sporozoite-infected mosquitoes and parous vectors in the intervention and control group over the study period

Assess the malaria vectors susceptibility levels to permethrin using standard WHO methods before, during and after the study.

In addition, data collected from this study will be used to parameterize models which will allow simulation of effects of PIC against malaria transmission under specific vector bionomic ecologies (indoor, outdoor and/or day-biting and insecticide resistance status) and disease transmission dynamics (e.g. baseline transmission intensity) utilizing specific data to better inform where PIC are expected to provide protection beyond the study site [56].

7.6 Methods

7.6.1 Type of study and design

A prospective, cluster-randomized, double blind, placebo controlled trial study design will be used to measure the impact of PIC and LLINs on clinical malaria, in all study participants in the study area relative to use of LLINs and a placebo impregnated clothing. The intervention will be applied at the community-level and the unit of randomization will be clusters. The study will be conducted for three years, where the first year will be used to establish the baseline malaria incidence and prevalence, entomological and socio-demographic indices in the study area. The two subsequent years will be used to capture variation in malaria transmission intensity between the treatment groups and cumulative effects of PIC on vector populations between the intervention and control group. A cluster-randomized design was used to prevent, intra and inter-household diversion of mosquitoes from compliant to non-compliant study participants [32, 57]. Also, the study aimed at measuring the community level effect of the intervention. The clusters will be selected from villages in Kilombero

district, in Southern Tanzania (Figure 1). All households in all the clusters will be recruited into the study and will be offered an LLIN per sleeping space at no cost to the recipient to enable all residents to benefit from this intervention and to minimize imbalance in use of malaria control interventions between intervention and control group of the study. This will also protect the community from higher than normal mosquito exposure as a result of potential diversion of mosquitoes from the intervention clusters as permethrin is known to have some repellency [32]. The clusters will then be randomized into two equal groups; all households in the clusters in one group will be assigned the PIC (intervention clusters) and those in the other group will be assigned the placebo-impregnated clothing (control clusters).

Both the permethrin and placebo will be packaged in identical bottles, which will have unique identification codes (UIC), identifying the contents of the bottles as having permethrin or placebo, which will be used for blinding the treatments and will only be known to the industry partner supplying the treatments.

To assess whether PIC and LLINs combined provide greater protection against clinical malaria in the community compared to LLINs and a placebo, 3000 study participants, as estimated from sample size calculations, will be recruited and enrolled into the study, after informed consent is requested and obtained from the household heads and household members above 13 years of age. The study participants will be followed for two years and will be screened for malaria by active and passive case detection every month. If malaria is detected by RDT, the subjects will be treated as per Tanzanian guidelines for each malaria infection [58]. Blood will also be collected using blood spots on filter paper for malaria PCR diagnosis [59], which will be carried out at the IHI laboratories. All members in the study-enrolled households will be instructed to seek treatment at dedicated study health facilities for any febrile

illness in between scheduled visits to test for malaria. Treatment at the dedicated study health facility will follow the national policy guidelines for malaria treatment [58].

Entomological end points will be monitored in the first year before intervention implementation to establish baseline entomological indices and in the two subsequent years after intervention implementation, in both the intervention and control clusters to assess the effect of the intervention (PIC) on vector density, parity, sporozoite and man biting rates. Adult sampling will be assessed using medically supervised HLCs. An independent entomological investigator will conduct entomological evaluations of the PIC so as to minimize the risk of premature unblinding of the epidemiological study team. The entomological investigator will conceal the assignment of product codes; GPS coordinates and cluster numbers before sending data to the study team (internal and external statisticians as well as study P.I) for analysis.

7.6.2 Study area

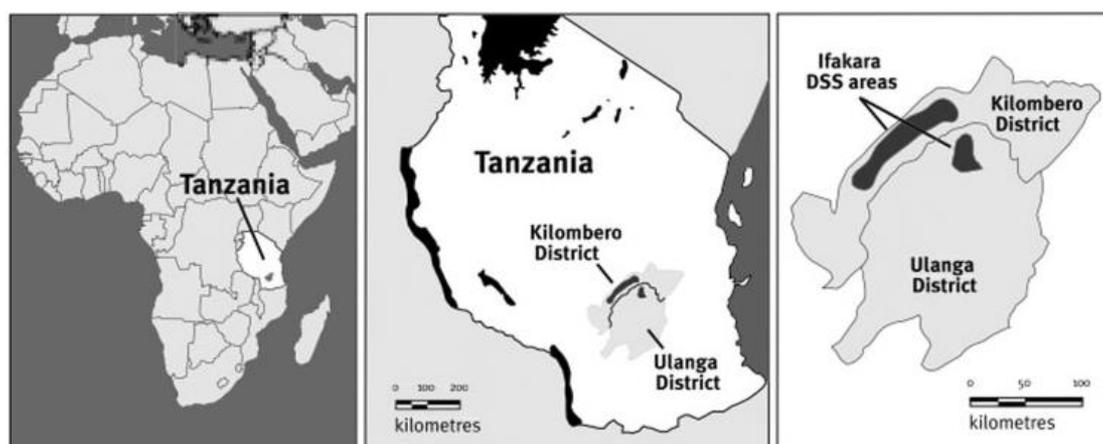


Figure 7:1 Map showing the planned study area [source: INDEPTH Monograph [60]]

This study will be conducted in, Ulanga district, Southeast Tanzania, located at 8.195°S and 36.259°E. The district experiences an annual rainfall range of 1200mm-1800mm and an average temperature range between 20° C. and 32.6 °C.

Entomological surveys conducted in 2011 identified *An. arabiensis* as the major malaria vectors in the study area [26]. Malaria transmission occurs all year round, with the month of November through April experiencing the highest transmission due to the long rainy season. In 2011, a cluster randomized clinical trial reported malaria cluster incidence rates of 0.06 in the control arm of the study [61]. The study area has experienced extensive malaria intervention education and implementation and therefore has the infrastructure related to malaria research already set up, such as health facilities and trained community health workers [26, 61-65]. Bed net coverage in the study in the study area is estimated to be 60-80 % [66].

7.6.3 Sample size and power calculation

Sample size estimates have been based on malaria incidence rates of 0.06 cases/person/year determined from a cluster randomized clinical trial, conducted in the study area in 2011 [61], Assuming that the PIC will have a PE of 30% against malaria incidence, it is estimated that to observe this effect at the two-tailed 5% significance level, with 80% certainty, and an inter cluster correlation of 0.25, 10 clusters of 30 households each per treatment group will need to be followed up for two years. A further 20% of the households will be recruited to account for loss to follow up (LTFU). Therefore a total of 600 households with ~ 5 members each or a total of 3000 individuals will be recruited into this study. However, as estimates of malaria incidence used for sample size estimation were based on data collected in 2011, a baseline malaria incidence survey will be conducted throughout the first year of the study, where all malaria cases from all health facilities recruited into the study

will be collected through passive case detection to determine a more valid malaria incidence rate in the study area. The study sample size will then be adjusted appropriately at the end of the first year of baseline data collection. (Appendix 1: Sample size calculation)

7.6.4 Randomisation

The unit of randomization will be the cluster made up of 30 households each. All 20 clusters will be assigned numbers. A random number generator will then be used to randomly assign treatments to the clusters, where the first number generated will be assigned to receive PIC and the second number generated will be assigned the placebo-impregnated clothing. This progression will be repeated until all 20 clusters are randomized to receive the PIC or a placebo-impregnated clothing in addition to an LLIN provided per sleeping space to all enrolled households. The PI will be responsible for randomization of clusters to the treatments. An attempt will also be made to stratify each cluster by the presence of a study recruited health facility. Stratification by health facilities will minimize the bias brought about by access to health services, which might confound the results of the outcome.

7.6.5 Blinding

Industry partners will manufacture and package the permethrin and placebo in identical bottles distinguishable only by a 3-digit unique identification code (UIC), identifying the contents as having permethrin or placebo. As permethrin is odourless, the manufacturer will ensure that the placebo is also odourless and similar in consistency as the permethrin. The manufacturer will also ensure the delivery of these bottles to the IHI. The code identifying the contents of each bottle will be kept strictly confidential by the manufacturers to ensure that neither the study investigators nor

participants are aware of the product assignment. Only the study monitor and manufacturer will have access to the code sheet that identifies the permethrin or placebo for routine study monitoring. However an extra list of the UIC will be stored in a secure location at the study site with limited access to the site PI who will be instructed to use it only in case of emergencies, (such as severe adverse events).

7.6.6. Controlling for confounders

Treatments will be assigned at the cluster level. All households within each cluster in the intervention group will be randomly assigned the PIC while all houses within each cluster in the control group will be assigned the placebo impregnated clothing. This will minimize the chances of diverting mosquitoes within and between households if treatments are assigned at individual or household level [32].

Analyses will follow a pre-defined analysis plan developed at the inception of this study. The investigators and statistician will be blinded throughout the analysis of the study.

Baseline socio-demographic data collected in the first year will be analyzed, and if a difference in factors that are likely to confound the treatment effect is found between the two treatment groups, then that analysis of the outcomes will be stratified by these variables.

Baseline malaria incidence data will be collected from all health facilities in the study area recruited into the study, using passive case detection to assess whether there are any imbalances in malaria rates between the intervention and placebo group and analysis of the final findings of the study adjusted accordingly.

Investigator bias will be reduced by use UICs on the permethrin and placebo bottles supplied by the manufacturer.

In addition, entomological and efficacy data of the PIC will be conducted by an independent entomological investigator, to minimize the chances of unblinding the investigators collecting the epidemiological data. This data will then be re-coded by an independent statistician before analysis where all investigators will be involved. Volunteers sampling adult mosquitoes will be rotated between households to minimize mosquito collector bias. Treatment allocation process will be randomly done and closely monitored by the P.I to minimize treatment allocation bias.

7.6.7 Entomological data collections

A sub-study will be conducted to assess the impact of PIC on the entomological endpoints of the study. The PIC will be evaluated against target vectors species in the study area to determine the duration of efficacy of the PIC under semi-field and field conditions.

7.6.7.1 Treatments

Permethrin-impregnated clothing (PIC)

Permethrin-impregnated clothing will be developed by impregnating clothes belonging to study participants with permethrin purchased from local industries. The permethrin target application rate will range from 0.25g/m² to 0.5gm/m², depending on the least application rate that is found to be the most effective from the semi-field and field efficacy experiments, so as to minimize the exposure of participants to permethrin. This range meets the WHO specifications for impregnating clothes with permethrin.

(<http://apps.who.int/disasters/repo/13389.pdf?ua=1>)

Long lasting insecticidal nets

The long-lasting insecticidal nets (LLINs), (insecticide incorporated into filaments) distributed during this project will be Olyset Nets, from Sumitomo

Chemicals that meets WHO specifications with permethrin incorporated into the polythene fibers at 2% w/w giving adequate release of permethrin for up to five years. Participants will be offered the “family” rectangular (160cm wide x 180cm l x 150 cm h) model.

http://www.who.int/whopes/Long_lasting_insecticidal_nets_Aug09.pdf

Packaging and labelling

- The permethrin will be provided in suitable plastic bottles that will be logistically manageable, especially for transport to the field sites for impregnation on a monthly basis. The containers will be labelled with the manufacturer’s instructions in Swahili and the unique identification code (UIC) identifying the contents of the bottles as permethrin or placebo.
- Olyset nets will be supplied in individual sachets labelled by the manufacturers and they will be distributed to participants in pre-opened individual sachets.

Product storage and stability

- The permethrin and placebo bottles will be stored at IHI until the day of impregnation, when they will be transported to the designated clusters for impregnation of participant clothing. They will be kept in tightly closed original shipping containers, that will provide a dry, cool and well-ventilated place accessible by authorized personnel only (study team) and away from food, drink and animal foodstuff.
- Olyset LLINs will be stored at ambient temperature in locked rooms at the IHI.

7.6.8 Intervention application

7.6.8.1 Impregnation shed

After the baseline survey, recruitment of households into the study and formation of clusters, impregnation sheds will be put up in each cluster. Large, flat concrete troughs will be constructed in designated areas in each cluster, with drainage into a pit latrine with a soak pit. This way the wastewater from cloth impregnation containing permethrin will be drain into the pit latrine and eventually seep into the ground through the soak pit. Permethrin binds to soil and sediment and does not leach into soil and is therefore unlikely to contaminate ground water [67]. Permethrin is also known to be degraded by micro-organisms, hence the disposal into the pit latrine [68]. This method of disposal will be used as its similar to burying, the recommended method of disposing permethrin waste water [69] and was chosen because apart from providing safe disposal method of permethrin, it will also benefit the community members in the study area with pit latrines. The WHO also recommends that wastewater from permethrin net impregnation be disposed into pit latrines.

http://www.who.int/water_sanitation_health/resources/vector088to118.pdf

Caustic soda will be poured into the pit latrine to help in hydrolyzing the permethrin to safe levels.

<http://www.inchem.org/documents/hsg/hsg/hsg033.htm#SubSectionNumber:>

4.5.1

7.6.8.2 Impregnation of study participant clothing

Apart from the UIC, the permethrin and placebo bottles will be labelled with their respective cluster number at the IHI as per randomization. The study participants from households in each cluster will be asked to bring all of their clothing to the

impregnation shed located within that cluster for impregnation. The clothing will then be impregnated with the appropriate amount of permethrin that will enable the permethrin to remain effective for one month assuming two washings per week, as determined from semi-field and field experiments following an outlined SOP. The study participants will also be advised to wash their clothing at the impregnation sheds to minimize contamination of the environment. Free detergent will be offered free of charge to all households washing their clothes at the impregnation shed to encourage compliance. The study participants will also be educated on the dangers of poor disposal of permethrin. A field worker will also be stationed daily at the impregnation shed within each cluster to record the number of households washing their clothes at the impregnation shed. Those households not observed/recorded at the impregnation shed for utmost two weeks will be visited and to determine why they did not use the impregnation shed for washing. Further, the ten-cell unit leader (TCUL) of each ten-cell unit (TCU) will be recruited to report any cases of washing away from the impregnation shed.

Requirements

The study team impregnating the clothing will be required to have:

- Plastic gloves
- Measuring vessel/cylinder
- Dipping/mixing bowl
- Soap
- Water
- Emulsifiable concentrate of treatment (either permethrin or placebo)
- Plastic sheet with a large surface area
- Large plastic disposal containers

Procedure

- The area of clothing to be impregnated will be calculated
- The volume of water absorbed per meter square of clothing will then be determined
- The target application rate of the least amount of permethrin providing the greatest efficacy against mosquito bites as determined from semi-field and field experiments will be applied.
- The emulsifiable concentrate formulation of the treatment will be mixed with water to achieve the target application concentration required for treating a square meter of clothing and only enough solution to impregnate the clothing presented for treatment will be prepared to reduce the amount of excess solution to be disposed.
- The clothing will be dipped into the solution and any excess solution wrung out
- The permethrin-impregnated clothing will be dried under a shade by spreading them horizontally on a spread out plastic sheet to prevent the permethrin depositing onto the soil and being washed off by surface run off into streams. Drying the clothing horizontally will also prevent patchy impregnation that might occur if clothing is hung vertically.
- After impregnation, study team will wash the dipping bowls and hands with soap and put all empty permethrin bottles and remaining insecticide in the large disposal plastic containers to be transported to the IHI for incineration.

- Study participants will be instructed to wash the permethrin-impregnated clothing up to twice per week to avoid loss of permethrin from clothing as a result of frequent washing

The participants will be asked to bring their clothing at the beginning of every month for impregnation at the impregnating shed constructed in each cluster. Therefore, ~ 150 individuals will present their clothing for impregnation by an appropriate number of trained field workers every month in each cluster.

The impregnation schedule will be done according to Figure 2.

Figure 7:2 Flowchart showing impregnation of clothing and study participants follow up

7.6.8.3 Long lasting insecticidal nets (LLINs)

The study team will distribute the LLINs at no cost to the participants. In advance of distribution, the number of beds/sleeping places in each household will be counted.

Distribution will be at the household level to the household head or representative to achieve coverage of one LLIN per bed/ sleeping place. The bags that LLINs are delivered in will be cut open before issuance to the household head/ representative to reduce opportunities for sale of the nets. In order to maximize compliance, the study team will hang the LLINs in the households. Net use will be monitored on a monthly basis during active case detection. The study participants will be sensitized on the correct net use and maintenance.

7.6.7 Monitoring for compliance

To assess compliance, study team field workers will be assigned households within the cluster where they will monitor use of PIC. The number of households assigned will be subject to logistics, i.e. how many households a single field worker can effectively oversee and funds available to hire an adequate number of field workers. Monitoring of intervention use will be conducted at the beginning of every month during cloth impregnation and during active case malaria detection two weeks after impregnation of the PIC. Random spot checks to assess whether the participants are compliant with this intervention will also be conducted by the field workers to ensure that households are not simply complying only when blood smear sampling and cloth impregnation will occur. Therefore compliance to use of PIC will be evaluated

fortnightly as well as through random spot-checks. Non-compliant households/participants will be recorded and excluded in the final analysis.

7.6.8 Treatment of study participants

During the active case detection, artemether–lumefantrine (Co-Artem) will be given to any participant with a temperature $\geq 37.5^{\circ}\text{C}$ or a history of fever in the past 48hrs, with *P. falciparum* parasites detected by RDT (SD Bioline Malaria Antigen Pf/Pan™ SD, RDT). Iron supplementation will be given to any participant with anaemia (Hb < 9 g/dL) measured using the HemoCue instrument. Any participant treated for malaria will be asked to attend the nearest health facility if no improvement/recovery is made within 48 hours. Participants with severe malaria will be referred to Ifakara District Hospital for treatment. Also during the study, participants will be encouraged to visit the nearest health facility in case of febrile illness in between scheduled visits. Study participants with a positive RDT will be treated following Tanzanian treatment guidelines with Co-Artem [58], while those with anaemia (Hb < 9g/dL) will be given iron supplementation. Treatment for other conditions will be carried out in accordance with Tanzanian national guidelines.

In case of a severe adverse effect related to the study, study participants will be referred to Ifakara District Hospital and treated according to the medical judgment of the local physician. The study will pay for transportation and any treatment required due to a study-related AE.

7.6.9 Selection of participants

Study participants will be recruited during baseline household enrolment using pre-defined inclusion/exclusion criteria. All community members in the study area will be eligible for inclusion into the study. Enrollment of study participants will occur at the household level. All participant households will be assigned household identification numbers (HIN). The aims and objectives of the study will be explained to the household head or parent/guardian and all present members of the participating household in local dialect, using local community health workers (CHWs) as needed and outlined in the informed consent form. All household members of the participating household will be assigned a unique identification number, (UIN). The name and UIN of each household member, subject to consent from the parent/guardian and all participating household members above 13 years old, will be recorded on a participant enrollment ledger. A Personal digital assistant (PDA) (Nanjing Corewise IC-7 Tablet PC, A370) with a biometric finger print scanner will then be used to scan the fingerprints of all household members of the participant household and link the each fingerprint with the respective HIN and UIN. Each study participant will be given an identification card with only their UIN but no name identifying the study participant. Informed consent will be given by each household head and each participating member above 13 years signing two informed consent forms: one for malaria testing and treatment and one for storage of blood samples (which will be used for PCR laboratory based parasite detection at the IHI laboratories).

7.6.10 Inclusion and exclusion criteria

Recruitment of each study participant will be according to the specific criteria listed in Table 7.1

Table 7:1 Outline of the inclusion/exclusion criteria of study participants

Inclusion Criteria	Exclusion Criteria
Household members sleep in cluster >90% of nights during any given month	Household members sleep in cluster <90% of nights during any given month
Household members have no plans for extended travel (<1month) outside of home during study	Household members have plans for extended travel (>1month) outside of home during study
Household members not participating in another clinical trial investigating a vaccine, drug, medical device, or a medical procedure during the trial	Household members participating or planned participation in another clinical trial investigating a vaccine, drug, medical device, or a medical procedure during the trial
Provision of assent/informed consent form signed by the study participant >13 years and by the household head or another legally acceptable representative for participants < 13 years	No provision of assent/informed consent form signed by the study participant > 13 years and by the household head or another legally acceptable representative for participants <13 years

7.6.11 Withdrawal of participants

Study participants and households are free to withdraw from participating in the study at any time without any prejudice.

7.7 Summary of study schedule

7.7.1 Introduction of study

Consent to conduct the study will be sought from the District Executive Director (DED) and the District Medical Officer (DMO). A letter explaining the study will be drafted and a representative from the study will discuss the study aims and objectives and address any questions arising with the DMO and DED.

After informed consent has been obtained from the DED, then village sensitization will begin. Representatives from the project will visit the study area to discuss the study and its objectives with the Village Executive Officers (VEO) and other local leaders as well as deliver copies of the letters sent to the DED. Information regarding the study area, like number of households in the study area and average number of household members will be obtained from the VEO and TCUL. An appointment for a community meeting will then be organised, with the help of the VEO and TCULs, where the project will be introduced to the community members in the study area. Posters and the social marketing team from IHI will be used to announce and sensitize the community on the dates of the meetings.

7.7.2 Community sensitization

Local leaders, including the VEO and TCULs will be the focal people used to endorse the study. Community sensitization meetings will be coordinated with the local District Health Management Team (DHMT) (or similar health authority present in the study area) and local community leaders. Refreshments will be provided and some light entertainment from members of the local arts college and IHI social marketing team will be organised to encourage attendance. A study team member, experienced in community education will deliver key messages. Information brochures detailing

key messages and including feedback from community meetings will be prepared and distributed during these meetings.

The sensitization meeting will outline the study objectives, aims and procedures. The community members will be informed on the allocation procedures of the PIC and placebo-impregnated clothing between the study groups. The community members will also be sensitized on the right to withdraw from the study at any time without prejudice. A description and demonstration of how to impregnate clothing will also be conducted during this meeting. All questions and concerns of the community members will then be addressed after which the meeting will be closed.

7.7.3 Training of health workers

Health facility and CHWs will be trained on safe blood draw techniques to determine malaria infection in training sessions conducted by the study team with the help of an experienced phlebotomist from the Ifakara District hospital. The CHWs, including the clinical (CO) employed by the study, will also be trained to recognize signs and symptoms of uncomplicated and complicated malaria as well as AEs caused by malarial drugs and permethrin. Additional training for RDT use and interpretation of results will be conducted with the help of experienced health workers from the Ifakara District Hospital. Field technicians will receive additional training on standard operating procedures (SOPs) of human landing catches and impregnation of clothing with permethrin that will be carried out during this study.

7.7.4 Household enrolment

During household recruitment visits, the study aims and objectives will be explained to the household head and members present at the time of the visit prior to requesting informed consent to recruit the household into the study. If informed consent is obtained, the GPS coordinates of the participant household will be recorded using a hand held GPS receiver (Garmin eTrex Legend® H) and a HIN will be stapled on the doorframe. The HIN will be used to link all clinical, entomological and intervention allocation data. Participants will be informed that they are free to withdraw their participation from the study at any time and for any reason without prejudice. Socio demographic household data collected will include: household construction materials, age and gender of household members, education level of household members, asset ownership and presence and density of domesticated animals. Knowledge, attitude, practice and perceived effectiveness of impregnated clothing data will also be collected. These data will be collected using a PDA with a biometric fingerprint scanner. The fingerprints of each household member will be scanned and assigned a UIN, which will be linked, to the HIN.

The census will be conducted from cluster to cluster using a team of 20 field workers, overseen by PL. The project will establish a base at the Village Headquarters where all CHWs and field workers will be allocated tasks and equipment will be stored. The PL will conduct regular spot checks to ensure that houses have been correctly enumerated and data recorded is consistent for a sample of households. Data will be checked at the end of the cluster census in case some houses have to be repeated due to data inconsistencies. Once all data is assured, the next cluster census will begin.

7.7.5 Cohort Follow-up

All study participants will be tested monthly, throughout the 2-year follow-up period, for malaria, regardless of symptoms (active surveillance). A PDA with a biometric fingerprint scanner, populated with the socio-demographic data of the study participant households as well as the HIN, UIN and GPS coordinates of the household will be used to collect the data during the household visit. Each study participant's history and symptoms will be recorded and results of the malaria diagnosis using RDT will be entered against the participants UIN in the PDA. This progression will be repeated for all household members. If a household member is absent on the first visit, a second visit will be attempted to collect clinical data. If still absent then that study participant will be excluded from the calculation of person-time for two weeks. Study participants positive for malaria parasites during active case detection will be given ALU (Co-artem), the first line drug for malaria treatment in Tanzania [58, 70] by the CHWs conducting the malaria diagnosis.

Participants will also be passively screened for malaria infection 2 weeks after active screening during the clothing impregnation exercise, with only those participants having a history of fever (fever $\geq 38.0^{\circ}\text{C}$) or manifesting symptoms at the time of this exercise being tested for parasite diagnosis (symptomatic screening). Study participants positive for malaria parasites during passive case detection will be given ALU (Co-artem), the first line drug for malaria treatment in Tanzania [58, 70]. Data will be captured in the same way as the active case detection using PDAs. Study participants will also be screened for anaemia using the HemoCue instrument during active case malaria detection. Study participants will therefore be screened for malaria fortnightly and once every month for anaemia. In addition, all participants will be

instructed to seek treatment at dedicated study health facilities, in case of febrile illness in between scheduled visits. A clinical officer and a nurse, both trained in malaria diagnosis using RDTs and collection of blood spots on filter paper for PCR diagnosis will be employed at each at each study recruited health facility. They will also be trained on recognizing the signs and symptoms of severe malaria cases and AE events related to permethrin for referral to the Ifakara District hospital. At this visit to the health facility, the participant will be examined and if history of fever or other signs or symptoms associated with malaria are present, the participant will be tested using RDT and if found positive, treated as per the Tanzania national guidelines[58, 70]. All study participants, will be given a card with their UIN to present at the clinic to receive screening and treatment if symptomatic. The card will contain the UIN identifying the participant, HIN and cluster number that the participant comes from as well as the treatment code assigned to that participant. The UIN will be used instead of the participant name to record of passive malaria detection of participants attending the health facilities. A PDA will be supplied to all study dedicated health facilities for collection of clinical data. Field workers will collect the captured information and upload from the PDA, via the mobile network to the central database at the IHI.

7.7.6 Malaria incidence determination

To determine malaria incidence, subjects will be followed up for malaria infection. In case of an infection, the study participant will be treated according to outlined national guidelines [58, 70] and follow-up continued (with each infection treated). Study participants infected with malaria will be excluded from the calculation of person-time for a period of one month. Trained CHWs will carry out the malaria diagnosis in their assigned clusters. Loss to Follow Up (LTFU) will be based only on

a study participant or household voluntarily leaving the study. A subject missing sample points (collection of blood samples to test for malaria, during active case detection) will be excluded from calculation of person-time for two weeks. All study participants retained in the cluster will be followed for a minimum period of 2 years to capture seasonal variations in malaria infections between the two study groups. At each monthly scheduled visit, blood samples will be taken for RDT diagnosis and blood spots, for PCR analysis. Malaria positive subjects, tested by RDT, will be treated using ALU (Co-Artem), the first line drugs for treatment of malaria in Tanzania [58, 70].

7.7.8 Passive Case Detection of Malaria episodes

Passive case detection for malaria will be maintained throughout the transmission season. Participants will be instructed to attend the nearest health facility to their household in case of febrile illness. The CO will perform the diagnosis of malaria using RDT and treatment of malaria if positive. The CO or nurse will also collect blood spots on a filter paper to be collected later by the field workers for PCR analysis at the IHI laboratories, at least once a week

7.7.9 Clinical data collection

7.7.9.1 Malaria diagnosis

Rapid diagnostic test (RDT) kits will be used for field screening and treatment of malaria infection during active and passive follow up of study participants. In addition to RDTs, blood spots on filter paper will also be collected for PCR analysis. Blood spots will be dried and stored under desiccant until they are transported to the IHI laboratories for PCR analysis at the end of each week after which they will be stored at up to -80°C. All blood spots will be processed using PCR techniques for detection

of malaria parasites [71]. A sample, 20% of all malaria positive RDTs used every week will be analyzed against their respective PCR blood spots collected. Discordant RDT and PCR results (positive RDT but negative PCR) will prompt re-training of CHWs in using RDTs. Used RDT kits will be stored in a locked shipping container at IHI until the data is completed and locked, then the samples will be destroyed on-site in the IHI incinerator. Blood spots sent to the IHI laboratory for PCR-based malaria diagnosis will be stored and destroyed according to IRB requirements and restrictions.

7.7.9.2 Malaria treatment

Malaria treatment in the field will be based on a positive RDT. Treatment of malaria positive study participants during follow-up visits will follow national policy guidelines on treatment of malaria [58, 70]. In case of severe malaria symptoms, the subject will be referred to the Ifakara district hospital for evaluation and treatment. The study will pay for transportation and treatment. In case of AE experienced as a result of participating in the study, the subject will be transported to Ifakara district hospital and treated according to the judgment of the local physician at study cost.

7.7.10 Entomological data collection

The evaluation of entomological end points will be carried out in both the intervention and control clusters to facilitate identification of the entomological correlates of using PIC.

7.7.11 Human Landing catches (HLC)

Human landing catches will be performed in the first year to establish the baseline entomological endpoints. The HLCs will then be conducted over the study period (2 years) in the intervention and control groups to evaluate the effect of the PIC on the entomological correlates. The entomological correlates of year one will be compared

to those of the second and third year in the control group, and if a difference is observed, this difference will be adjusted for when analyzing the findings of the intervention and control group. Human landing catches (HLCs) will be performed every week to measure anopheline-landing densities.

Two households each, from the intervention and control clusters will be randomly chosen at the beginning of every week for entomological sampling of mosquitoes. Mosquitoes will be collected from these households using all night HLCs. The entomological study will be conducted by an independent study entomologist to ensure that the rest of the study team remain blinded to treatment allocation. A team of four field workers trained on HLC will conduct these collections. The first pair of field workers will collect mosquitoes from 6 p.m. -12 a.m. The second pair will collect mosquitoes from 12 a.m. to 6 a.m. The collections will be conducted twice a week, for every week through out the study period and in both the intervention and control group simultaneously. Volunteers will alternate indoor and outdoor positions during each night of collection, throughout the study period. All mosquitoes landing on volunteers (both anophelines, and culicines) will be held in hourly marked paper cups so that it will be possible to determine the number of mosquitoes collected in each hour, from the first through to the sixth hour, and from the sixth to the 12th hour. The sampled mosquitoes will then be transferred to the field office at the village headquarters, where they will be killed using petroleum vapours. All mosquito sampled will be sorted into anophelines and culicines. Culicine mosquitoes will be identified to species level using appropriate keys [72]. Mosquitoes identified to be anophelines will be transported to the IHI lab where PCR speciation will be conducted [73]. Data collected will be recorded by: mosquito species, household

identification number (HIN), date of collection, sampling hour, location of mosquito collector (indoor/outdoor) and name of collector.

7.7.11.1 Sporozoite infection rates

All anophelines captured from the HLC will be processed at the IHI laboratory using enzyme-linked immunosorbent assay (ELISA) techniques for detection of sporozoites of *Plasmodium* parasites [74]. A sample, (20%), will be processed at the NIMR laboratory as a quality check of ELISA techniques at the IHI laboratories.

7.7.11.2 Parity rates (Age structure)

A subset of samples, (20%), of each morphologically identified adult anopheline species from HLC collections will be dissected for parity determination (nulliparous vs. parous) following standard WHO protocols [75]. Adult mosquitoes will be sorted in the field office following completion of HLC collections. Approximately 20% of females will be dissected by hour, indoor/outdoor location, collector's identity, (name) and HIN. When hourly collections are ≤ 10 , a minimum of 2 specimens will be dissected and characterized. For larger collections up to 30 mosquitoes will be dissected for parity determination.

7.7.11.3 Vector species determination

All adult anophelines captured will be identified to species level using PCR [72, 73]. Other mosquito genera identified will be counted and stored for potential future processing.

7.7.11.4 Vector insecticide resistance

Baseline vector permethrin resistance will be assessed in the first year prior to implementation of the intervention in the study groups using standard WHO methods for measuring insecticide resistance and susceptibility [76]. Mosquitoes tested will be

assessed for molecular species identification post insecticide resistance assays using PCR [73]. PCR assays will also be conducted to detect known resistance alleles as quality assurance method for standard WHO resistance evaluation bioassays [77]. Assessments will be repeated mid and post study, to determine the effect of PIC on vector species-specific permethrin resistance levels between the study groups.

7.8 Data Management and processing

7.8.1 Data collection

Data will be collected and recorded according to UIN and HIN. Socio-demographic data (age, place of birth, and gender), household census data (household construction materials), travel habits, past medical history, drug allergies and drug history, will be collected during household enrolment subject to consent. Knowledge, attitudes and practice of the household head/members in relation to PIC will also be recorded during household and participant enrollment. Data on malaria infection (clinical data) will be collected every two weeks by alternating active and passive case detection using RDTs and blood spots throughout the study period. The HIN and UIN codes of all study participants tested will be recorded in the subject case report forms (CRF), uploaded in the PDA. All, adverse events (AEs) experienced by study subjects, whether or not related to the study, will be captured in AE forms throughout the study period also uploaded in the PDA. Data on concomitant drugs use, such as antibiotics (Septrin), that directly affect malaria transmission, received during the intervention period will also be recorded. All this data will be linked by the HIN of the households and UIN of study participants in the PDA.

7.8.2 Data capture

Data will be collected using standardized forms on PDAs with a biometric fingerprint scanner. In case of PDA failure, paper based data collection will be used as back up. The PI will oversee the accuracy of all data entered on the PDAs/forms. The field workers will be trained and instructed on proper data input methods to ensure data integrity.

Collected data will be directly uploaded from the PDAs via the mobile network to a central database managed by a study data administrator at the IHI. The data administrator will check for data integrity and inconsistencies and advise if data collection should be repeated. After data entry, all data collected using paper data forms will be scanned and transferred to binders for storage. All the data collected electronically (PDAs) will be archived with a documented history of changes or corrections.

7.8.3 Data storage

All the data collected using paper format from the study subjects: history of illness, physical findings, consultations and laboratory results of will be stored in a secure storage facility in locked cabinets and will be maintained in compliance with IRB requirements. If these data are collected electronically, they will be stored in password secured databases at the IHI. The study PI will be responsible for retaining copies of the completed CRFs, in both electronic and paper formats. All raw and cleaned data forms will be archived in a central data warehouse.

7.8.4 Data validation

Data collected using paper forms will be double entered into a computer by two data entry clerks. These dataset will then be cross-referenced and errors and inconsistencies will be resolved by checking the data source forms, after which the dataset will be combined to produce a single data set. Data collected using PDAs, will be checked for quality before being incorporated into the central database.

7.9 Data analysis

An internal project statistician, as well as an external statistician, both blinded to the intervention allocation, together with study investigators will perform the data analysis, following a pre-defined statistical analysis plan.

7.9.1 Clinical data

An intention to treat analysis (ITT) will be performed on all data, after which, according to protocol (ATP) analysis excluding participants who were not compliant with the intervention or willingly withdrew from the study will be done. Person-time will be calculated as the number of months that the study subject was compliant with the interventions during the study. In case of malaria infection, the study subject will be excluded from the study for one month to avoid recording a single infection twice. If malaria data is not sampled from the study participant, after a second visit will be attempted, after which that participant will be excluded from the calculation of person-time for two weeks if unavailable for data collection. ITT analysis will include all months that the study participant was enrolled in the study regardless of compliance to the intervention. According to protocol analysis (ATP) will include only the person-time for which study participants were compliant with the intervention. A Poisson regression model, adjusted for intra-cluster variation by

random effects will be used for analysis. This model allows for repeated measures on a single individual (study participant). The results of the model will be presented as incidence rate ratios (IRR) of malaria between the intervention and control groups. All variables significantly associated with the intervention from univariate and multivariate analysis will be included in the model.

7.9.2 Demographic data

A principal component analysis (PCA) will be used to develop a socio-economic score for each household. Variable to be included in the PCA include, asset ownership, household construction materials, occupation and education level of household head.

7.9.3 Compliance with interventions

Compliance of participants will be compared between the intervention and placebo group using a chi-square test. LLIN and PIC use will be converted to proportions of use per two weeks and will also be included in the regression model for disease (malaria) incidence.

7.9.4 Entomological data

A Poisson regression model with a log link function and a random intercept will be used for analysis of entomological data. This model allows for repeated measures over time. The number of mosquitoes caught per hour will be the dependent variable, and time, position and individual will be used as independent variables in this model. Day, which accounts for abiotic factors will be fitted as a covariate and a random intercept will be fitted to account for over dispersion often seen in mosquito data.

7.9.5 Data dissemination

Study results will be disseminated to the scientific community through peer-reviewed journals and international conference forums with yearly progress reports distributed to key stakeholders. Summary reports will be generated by the PI and sent to the study sponsor on a monthly basis.

7.10 Potential risks and benefits

7.10.1 Risks

The risk of permethrin toxicity is minimal as permethrin has been approved for use as an insecticide by the EPA [36]. Permethrin has been used for an extended period of time to impregnate clothing by the US army for protection against arthropod bites in the field, [41], and has been shown to have low mammalian toxicity [41]. The least amount of permethrin demonstrating the most effective protection will be used to minimize unnecessary exposure of study participants to permethrin.

Antimalarial drug treatment will be provided according to national guidelines [58] and national first and second line antimalarial drugs will be used for treatment of all malaria infections. Medically trained personnel will treat all malaria cases. Adverse Events (AEs) or Severe Adverse Events (SAEs) related to permethrin or malaria treatment will be documented and reported to the PI and Institutional Review Boards (IRBs). The study CO will provide treatment, or, if severe, the participant will be referred to the Ifakara district hospital for treatment. Transport and treatment costs at Ifakara District hospital will be paid for by the study.

The amount of blood collected will be minimal: Blood samples by finger pricks will be drawn by trained personnel ensuring that these collections are done in as safe a manner as possible. WHO guidelines for blood collection will be followed [78].

7.10.2 Benefits

There will be no monetary incentives provided to the participants of this study.

However, the cost of research and blood tests will be covered by the study as well as provision of free treatment. If study participants will be required to attend scheduled visits at the study clinic, travel reimbursements or transport will be provided so that participants incur no cost of participating in the study. The study will also pay for any malaria drug, permethrin related AEs/SAEs or any other study related injuries.

Clinical trial insurance will be purchased for general liability and negligence protection. All study participants will receive free diagnosis and treatment for malaria if diagnosed at any point during the study. In addition all enrolled households will receive an LLIN for every sleeping space. All participants will be referred to a local clinic for any other illness. A pit latrine will also be put up in each cluster for community use at study cost.

7.11 Ethical considerations

7.11.1 Informed consent

Informed consent will be obtained directly from all adults participating in the study. For subjects <18 years of age, consent will be obtained from parents or guardians; in addition, personal child assent will be obtained for children who are >13 but <18 years and live with a parent or guardian. All consent forms will be translated into local languages, pretested on a sample of community members and back translated into English to ensure accuracy. The consent forms will then be amended accordingly into a final draft. During the consenting process, the study will be described and the consent form will be read to the household members. The consent form will detail the design of the study; outline the questionnaires to be administered and the blood draw

process. The purpose of collecting and storage of blood samples (to be used for laboratory based PCR malaria diagnosis) will also be explained to the household members and that no data on any other disease will be collected from blood samples. Each individual household member will be given an opportunity to ask any questions they might have in relation to the study, and if they agree, will be asked to sign the consent form. If they agree to have blood samples of household members stored, the household head will be asked to sign consent for blood sampling and long-term storage of samples. All households will be provided with signed copies of the consent forms after they have agreed to participate in the study. For households participating in entomological sampling, a script will be read to each household head or representative to explain the mosquito collection techniques and signed consent will be sought from the head of household. For incidence follow-up surveys, individual informed consent will be sought separately from parents/guardians and all eligible participants and household members >18 years.

Informed consent will also be sought from field technicians conducting the HLCs and impregnation of clothing with permethrin.

7.11.2 Confidentiality

Household identification numbers (HIN) and unique identification numbers (UIN) will be used to identify the study households and participants instead of names. All study related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff or in password protected databases. A UIN will be used to identify all laboratory specimens sampled and study data collected. Study participant names will only be used during baseline data collection after which assigned UINs will be used to identify study participants henceforth. Names of study participants or other personal

identifiers will be stored separately from study records. The database will be secured with password-protected access systems. Written permission from the participant will be required before any information regarding the said participant is released, except as necessary for the independent monitoring; representatives of other government and regulatory authorities, and/or site IRBs/ECs. All data will be stripped of personal identifiers (personal details removed and replaced with codes and GPS information blurred)) prior to any subsequent analysis and/or sharing after explicit permissions according to IRB approvals.

7.11.3 Study discontinuation

The study will be stopped on suspicion of potential harm to the study participants or the environment such as a large number of participants reporting an excessive number of adverse events.

7.11.4 Safety parameters

Permethrin-treated long lasting nets and permethrin-impregnated clothing have been fully evaluated by the WHO Pesticide Evaluation Scheme and approved for vector control and the products will be used in compliance with their recommended use and guidelines [36].

All study participants enrolled in the study will have access to malaria diagnosis and treatment according to Tanzanian National Treatment Guidelines and all visits to the health facilities will be recorded.

7.12 Safety oversight

The study CO will check all data regularly, at least once a week, and will inform the study PI of the numbers of malaria attacks and AE by cluster.

7.12.1 Adverse events

Any undesirable event that occurs to a study participant during the course of the study will be designated an adverse event (AE); i.e., any event occurring from the time of consent into the study until study ends (until the last follow-up visit for that specific participant whether or not that event is considered related to malaria treatment drugs, PIC, concomitant drugs and/or malaria infection detection procedure).

7.12.2 Serious adverse events

An AE will be defined as a serious adverse event (SAE) if it results in any of the following outcomes: 1) death, 2) life-threatening event – this means that the participant was at immediate risk of death at the time of the event and required immediate medical intervention. It does not refer to an event that hypothetically might have caused death if it were more severe, 3) prolongation of existing hospitalization or re-hospitalization once discharged, 4) persistent or significant disability/incapacity (a substantial disruption of a person's ability to conduct normal life functions) and; 5) an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

7.12.3 Patient management of adverse events

All adverse events will be treated as clinically recommended and treatment given recorded on the CRF in the PDA. If necessary, participants will be referred for specialist care at the Ifakara District hospital. The study CO will stop the study drugs or intervention if he/she determines that an AE is drug-or intervention-related and that stopping the drugs/ intervention is clinically indicated.

7.12.4 Adverse event reporting

The occurrence of adverse event will require prompt reporting to the study PI, IRB and oversight committees

These AEs will be reported by the site PI to the study sponsor as recommended in the IRB specifications. At consent, subjects will be provided with the local authority and study coordinator contact information in the informed consent form to use in case an adverse event occurs.

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Contribution made by others

Chapter 2: I did 80% of the work. I designed the semi-field and field experiments and drafted protocols under supervision from my supervisor Dr. Sarah Moore. I drafted the first manuscript with the help of my supervisor and wrote the final draft incorporating suggestions from co-authors.

Chapter 3: I did 70% of the work. I collected the field data, under supervision from Dr. Sarah Moore and with the help of field technicians. I received statistical supervision from Dr. Elizabeth Turner. I drafted the first manuscript with the help of my supervisor and wrote the final draft incorporating suggestions from co-authors.

Chapter 4: I did 80% of the work. I designed the semi-field and field experiments and drafted protocols under supervision from my supervisor Dr. Sarah Moore. I drafted the first manuscript with the help of my supervisor and wrote the final draft incorporating suggestions from co-authors.

Chapter 5: I did 70% of the work. I conducted the literature search and drafted the first manuscript under supervision from my supervisor Dr. Sarah Moore. Dr. Sarah Moore wrote the initial introduction to this chapter. I drafted the final manuscript with the help of my supervisor and wrote the final draft incorporating suggestions from co-authors.

Chapter 6: I did 80% of the work. I drafted the chapter under supervision from Dr. Sarah Moore. I incorporated suggestions from my supervisor in the final draft of this chapter.

Chapter 7: I did 70% of the work. I wrote the first draft of the protocol with the help of Dr. Sarah Moore. The final protocol incorporated suggestions from Dr. Sarah Moore

8 Appendices

8.1 Appendix 1: Stata output showing Eigen scores of each variable used in calculation of socio economic status of households

8.2 Appendix 2: Repellent KAP survey tool

8.3 Appendix 3: Sample size calculation

Stata output showing Eigen scores of each variable used in calculation of socio economic status of households

Variable	Comp1	Comp2	Comp3	Comp4	Comp5	Comp6	Comp7	Comp8	Comp9	Comp10	Comp11	Unexplained
education_~0	0.1991	-0.0767	-0.0634	0.6175	0.4955	0.4902	-0.1752	0.2258	0.0009	0.0283	0.0285	0
source_of_~y	0.3130	-0.0594	-0.0307	0.4565	-0.1299	-0.7048	0.1092	0.3599	-0.1776	-0.0508	-0.0009	0
source_of_~g	0.2245	-0.1412	0.1452	0.1974	-0.7354	0.4635	0.3175	0.0995	0.0156	0.0156	0.0529	0
roof_mater~s	0.5103	-0.2471	0.0057	-0.2689	0.1167	0.0511	0.0283	-0.0238	0.0355	-0.2133	-0.7361	0
flooring_m~l	0.4538	-0.1884	0.0624	0.1294	-0.0907	-0.1328	-0.3820	-0.6672	0.1539	0.1261	0.2853	0
walling_ma~l	0.4252	-0.1940	0.0390	-0.4822	0.2582	0.0598	0.2234	0.2965	-0.1294	0.0226	0.5676	0
bicycle	0.1966	0.5615	0.2079	-0.0523	-0.1147	0.0799	-0.3490	0.0953	-0.0798	-0.6540	0.1250	0
stove	0.0592	0.2794	0.5119	0.1660	0.2852	-0.0176	0.5745	-0.3958	-0.2414	0.0115	-0.0456	0
mobile_phone	0.2790	0.5201	-0.1849	-0.1177	-0.1004	0.0048	-0.1699	0.0616	-0.3734	0.6242	-0.1571	0
radio	0.0081	0.0486	0.6884	-0.0663	0.0170	-0.0908	-0.2152	0.3250	0.4853	0.3421	-0.0793	0
motorbike	0.2115	0.4126	-0.3939	0.0342	0.0591	-0.0467	0.3647	-0.0293	0.6977	-0.0011	0.0476	0

Community Perception of Repellents

Q1. Je wewe au mmojawapo kwenye kaya yako ameugua malaria kwa mwezi moja uliopita? Have you or anyone in your household suffered from malaria in the past one month?

1= Ndiyo Yes

2=Hapana No

3= Sijui Don't Know | _ |

Q2. Malaria ni nini? What is malaria?

Q3. Unafikiri malaria unasababishwa na nini? (Usimsomehe) What do you think causes malaria? (Do not read out the answers)

1= Ndiyo Yes

2=Hapana No

3= Sijui Don't Know

- | | |
|--|---|
| 1. Mbu Mosquito | _ |
| 2. Majira ya baridi au mvua Cold weather | _ |
| 3. Mafua Flu | _ |
| 4. Maji machafu Dirty water | _ |
| 5. Vinginevyo Other _____ | _ |

Q4. Je unajua jinsi ya kuzuia kupata malaria? Do you know how to prevent contracting malaria?

Q5. Ni maeneo gani mbu huzaliana? (Usimsomehe) Where do mosquitoes breed? (Do not read out the answers)

1= Ndiyo Yes

2=Hapana No

3= Sijui Don't Know

- | | |
|--------------------------------------|---|
| 1. Maji yaloyotuama stagnant water | _ |
| 2. Mashambani Fields | _ |
| 3. Mitoni Rivers | _ |
| 4. Kwenye vyoo Pit latrines | _ |
| 5. Kwenye takataka Dirty environment | _ |
| 6. Kwenye udongo Soil | _ |
| 7. Vinginevyo Other _____ | _ |

Q6. i) Je, wewe na wengine kwenye Kaya yako mnafanya nini ili kujizuia kuumwa na mbu? What do you or your household normally do to prevent yourselves from getting mosquito bites?

Q6. ii) Kwa nini munapendelea njia hii? Why do you prefer this method?

Njia(Method)	Ndiyo (Yes)=1 Hapana No=2	Bei Nafuu(Cheap)	Inapatikana kwa urahisi (Readily available)	Rahisi kutumia (Easy to use)	Ina uwezo zaidi(Effective)	Sababu Nyingine(Other) Andika(Write)
Kujifunika (Covering yourself)						
Kufukiza moshi (Smoky fire)						
Dawa za kupaka mwilini (Repellents on the body)						
Kupuliza dawa za kuua ukutani (Using insecticides)						
Kuchoma dawa za dukani (Burning mosquito coils)						
Kujaza mashimo yaliyo na maji ya kusimama (Fill puddles)						
Kufyeka maajani (Clear away bushes)						
Kutumia vyandalua (Use bed nets)						

Q7. Je wewe na wengine kwenye kaya yako mnatumia dawa za kijipaka mwilini ili kuzuia kuumwa na mbu? Do you or anyone in your household use mosquito repellents? (Kama hapana ruka hadi swali la Q.17) (If no go to Q.17)

|_ |

1= Ndiyo Yes

2=Hapana No

3= Sijui Don't Know

Q8. Dawa hiyo inaitwaje? What is the name the repellent? _____

Q9. Dawa hiyo inapatikana wapi? Where do you get the repellent from?

|_ |

1. Duka la dawa Pharmacy
2. Dukani Shop
3. Zahanati Dispensary
4. Kwinginepo Other _____

Q10. Dawa hiyo ni ya aina gani? What type of mosquito body repellent do you use? | _ |

1. Ya kupuliza Spray
2. Losheni Lotion
3. Mafuta ya kupaka Oil
4. Jeli Jelly
5. Nyinginezo Other _____

Q11. Dawa hiyo inajazwa kwenye nini? In what is the repellent packaged?

1. Chupa Bottle
2. Mkebe Tin
3. Tyubu Tube
4. Plastiki Plastic
5. Nyinginezo Other _____

Q12. Dawa hizo zinapakwa wapi? Where do you apply the repellent? | _ |

1. Mikono na miguu peke yake (Limbs only)
2. Mwili mzima (Whole body)
3. Sehemu za mwili pasipo na nguo (Exposed areas only)
4. Sehemu nyingine (Other) _____

Q13. Ni kitu gani kinachokupendeza kwenye dawa hizo za kufukuza mbu? What do you like about the repellent?

1= Ndiyo Yes

2=Hapana No

3= Sijui Don't Know

- | | |
|--|---|
| 1. Inanukia (Smells nice) | _ |
| 2. Ni nzuri kupaka (Feels good on the skin) | _ |
| 3. Ina rangi inayopendeza (Has a nice colour) | _ |
| 4. Inapatikana kiurahisi (Readily available) | _ |
| 5. Bei nafuu (Cheap) | _ |
| 6. inazuia kuumwa na mbu (Protects against mosquito bites) | _ |
| 7. Nyingine (Other) _____ | _ |

Q14. Unaupaka dawa hizo mara ngapi kwa mchana au usiku? How many times do you apply the repellent in a day/night?

| _ |

Q15. Unapaka dawa wakati gani? When do you apply the repellent? | _ |

1. Machweo (At sunset)
2. Kabla ya chakula cha jioni (Before supper)
3. Baada ya chakula cha jioni (After supper)
4. Kabla ya kulala (Before bed)
5. Nyingine (Other) _____

Q16. Kwa kawaida ni wakati gani unaoingia ndani ya nyumba wakati wa jioni? What time do you go into the house in the evening? | _ | _ |

Q17. Kwa kawaida unaenda kulala wakati gani? What time do you go to bed? | _ | _ |

Q18. Sababu zipi zinakufanya usitumie dawa za kupakaa mwilini ili kuzuia kuumwa na mbu? | _ |

Why don't you use mosquito body repellents?

1. **Hatujasikia** (I have never heard of them)
2. **Gharama** (Expensive)
3. **Haipatikani** (Not readily available)
4. **Hatuielewi** (Don't understand)
5. **9. Nyinginezo** (Other) _____

Q19. Je kama masuala ya hapo juu yakipatiwa ufumbuzi mko tayari kujaribu kutumia dawa za kupaka mwilini kuzuia kuumwa na mbu? (*Janibu kuwashawishi wajadili*) Would you be willing to use mosquito body repellents if the above concerns are addressed? (*Prompt them to discuss*)

Q20. Je, mngependa au mkotayari kujaribu njia nyingine za kuzuia malaria? (*Msomee kutoka orodha ya swali la Q6*).

Would you like or be willing to try additional methods to prevent malaria?

(*Prompt from list in Q61*) **Njia yapi?** If so which ones

C	Person years calcula	v	u	λ1	λ0	Treatment	Control	Effect size
10.11222222	2468.148148	1.96	0.84	0.042	0.06	0.042	0.06	0.3
Person-years								
$y = (v + u)^2 (\lambda_0 + \lambda_1) / (\lambda_0 - \lambda_1)^2$								
A	(v+u)	2.8						
	(v+u)(v+u)	7.84						
B	λ0+λ1	0.102						
C	λ0-λ1	0.018						
D	(C)(C)	0.000324						
y	A(B)/D	2468.1481						
Clusters								
$c = 1 + (v + u)^2 \left[(\lambda_0 + \lambda_1) / y + k^2 (\lambda_0^2 + \lambda_1^2) \right] (\lambda_0 - \lambda_1)^2$								
A		7.84						
E	B/y	4.133E-05						
	k	0.25						
F	(k)(k)	0.0625						
G	(λ0)(λ0)	0.0036						
H	(λ1)(λ1)	0.001764						
I	(G+H)	0.005364						
J	(I)(F)	0.0003353						
D	(C)(C)	0.000324						
	A(E+F(I))/D	9.1122222						
c		10.112222						
Households = clusters*person-years/household number								
Households 48.81514852								

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Student	SANGORO PETER ONYANGO
Principal Supervisor	MARY CAMERON
Thesis Title	CAN REPELLENTS PREVENT MALARIA IN TANZANIA

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	Malaria journal		
When was the work published?	26th April 2014		
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METHODOLOGY

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Use of a semi-field system to evaluate the efficacy of topical repellents under user conditions provides a disease exposure free technique comparable with field data

Onyango Sangoro^{1,2*}, Dickson Lweitojera¹, Emmanuel Simfukwe¹, Hassan Ngonyani¹, Edgar Mbeyela¹, Daniel Lugiko¹, Japhet Kihonda¹, Marta Maia^{1,3,4} and Sarah Moore^{1,3,4}

Abstract

Background: Before topical repellents can be employed as interventions against arthropod bites, their efficacy must be established. Currently, laboratory or field tests, using human volunteers, are the main methods used for assessing the efficacy of topical repellents. However, laboratory tests are not representative of real life conditions under which repellents are used and field-testing potentially exposes human volunteers to disease. There is, therefore, a need to develop methods to test efficacy of repellents under real life conditions while minimizing volunteer exposure to disease.

Methods: A lotion-based, 15% *N, N*-Diethyl-3-methylbenzamide (DEET) repellent and 15% DEET in ethanol were compared to a placebo lotion in a 200 sq m (10 m × 20 m) semi-field system (SFS) against laboratory-reared *Anopheles arabiensis* mosquitoes and in full field settings against wild malaria vectors and nuisance-biting mosquitoes. The average percentage protection against biting mosquitoes over four hours in the SFS and field setting was determined. A Poisson regression model was then used to determine relative risk of being bitten when wearing either of these repellents compared to the placebo.

Results: Average percentage protection of the lotion-based 15% DEET repellent after four hours of mosquito collection was 82.13% (95% CI 75.94-88.82) in the semi-field experiments and 85.10% (95% CI 78.97-91.70) in the field experiments. Average percentage protection of 15% DEET in ethanol after four hours was 71.29% (CI 61.77-82.28) in the semi-field system and 88.24% (84.45-92.20) in the field.

Conclusions: Semi-field evaluation results were comparable to full-field evaluations, indicating that such systems could be satisfactorily used in measuring efficacy of topically applied mosquito repellents, thereby avoiding risks of exposure to mosquito-borne pathogens, associated with field testing.

Keywords: Repellent, *Anopheles arabiensis*, Semi-field system, Efficacy, *N, N*-diethyl-3-methylbenzamide (DEET)

Background

Evaluations of topical repellent efficacy against blood feeding arthropods require standardized laboratory and field tests [1-3]. However, conditions in the laboratories are not representative of real life settings where repellents are used. Therefore, experiments carried out in the

laboratory may not accurately estimate the efficacy of repellents in the field [4]. Environmental factors such as temperature, humidity and wind speed, all of which affect the effectiveness of repellents, are controlled in the laboratory, but in the field these factors may fluctuate and affect repellent efficacy [5]. As a result, tests carried out in the laboratory ideally should be verified using representative field tests. On the other hand, field evaluations, albeit representative of conditions under which repellents are normally used, can expose volunteers participating in these experiments to mosquito-borne

* Correspondence: psangoro@ihi.or.tz

¹Ifakara Health Institute, Box 74, Bagamoyo, Tanzania

²Disease Control Department, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

Full list of author information is available at the end of the article

pathogens [6]. Therefore, there is a need to develop methods to test efficacy of repellents under representative user conditions while minimizing volunteer exposure to vector-borne diseases.

There are several techniques that have been proposed for testing topical repellents while reducing human exposure to mosquito bites. These options include: 1) use of synthetic mosquito attractants that mimic human volunteers [7]; 2) use of animals instead of human volunteers [8,9]; 3) use of *in vitro* blood feeding membrane [10-12]; 4) *In vitro* olfactometry [13]; and, 5) use of a semi-field system (SFS) [14,15]. Although techniques 1 to 4 are convenient because of their high throughput in screening of repellents and do not use human participants, they have well-documented limitations: as the skin is the site of action of topical repellents, and mosquitoes are attracted to cues produced by the host, different hosts will elicit varying degree of responses in the mosquito which will affect both duration and degree of repellency observed [8,10]. The use of *in vitro* blood-feeding membrane is unlikely to give similar results to repellents applied to human skin, as the feeding membrane used in these tests are structurally and physiologically different from the human skin and produce no odour [10]. Use of *in vitro* olfactometry, used mainly to test spatial repellents, is more suitable for screening purposes as it's used in confined spaces and shorter distances in the laboratory and results cannot be correlated to the field, where there are wide open spaces for the mosquitoes to forage [13]. The use of synthetic blends to test repellency has also proved unreliable as different repellent-blend combinations produced disparate results [7]. Use of SFS may overcome these shortcomings because efficacy tests can be performed in a large enclosure under ambient conditions, allowing mosquitoes to elicit similar behavioural responses as under field conditions. The other advantage of SFS is that it uses mosquitoes reared under laboratory conditions and therefore does not expose volunteers to potential mosquito-borne disease. The species, numbers and physiological status of mosquitoes used in the SFS are standardized to provide more controlled conditions and therefore reduce data variability associated with field studies. However, the effectiveness of SFS has not been evaluated against full-field conditions when testing topical repellents. This study examined whether tests carried out in a SFS would yield comparable results to tests conducted in field setting.

Methods

Study area

Semi-field evaluation of repellents was carried out at Ifakara Health Institute (IHI), Morogoro, Tanzania. The field evaluation of repellents was conducted in Mbingu

village, Ulanga district, situated 55 km west of Ifakara town at 8.195°S and 36.259°E. Rapid diagnostic test (RDT) results from passive case detection at a local clinic between December 2012 and July 2013 confirmed malaria incidence estimates from the village were 0.67 cases/person-years, (Jabari Mohammed Namamba, pers comm), only one-and-half years after the end of a national campaign to achieve universal coverage with long-lasting, insecticide-treated bed nets (LLINs) [16]. There is high malaria transmission all year round, with peak transmission occurring in the months of May and June after the long rains. The village experiences an annual rainfall of approximately 1,200-1,800 mm and an annual temperature range of between 20 and 32.6°C. The village borders an extensive field cleared for irrigation, which provides an ideal breeding site for malaria vectors [17].

Semi-field evaluations of topically applied repellents

The semi-field evaluation was carried out in the IHI SFS. A SFS is an enclosed environment, situated in the natural ecosystem of a target vector and exposed to ambient conditions necessary for the completion of the life cycle of the vector. It is made up of a greenhouse frame with walls of mosquito netting and a polyethylene roof, mounted on a raised concrete platform [14,15].

Mosquitoes

The mosquitoes used in these experiments were laboratory-reared *Anopheles arabiensis* (Ifakara strain, originally sourced from Sagamaganga village, Kilombero district in 2008) from the IHI insectaries. The larvae were fed on Tetramin® fish food and maintained at temperatures of $28 \pm 1^\circ\text{C}$. Pupae were placed in emergence bowls inside a $30 \times 30 \times 30$ cubic cm netted cage in a separate room where temperatures were maintained at $27 \pm 3^\circ\text{C}$ and relative humidity at 70-90%. A 10% glucose solution was supplied in the cages for the emergent adults. The insectary was maintained at 12:12 (light: dark) photoperiod, from 0600 hrs to 1800 hours (light period) and 1800 hrs to 0600 hrs (dark period). The mosquitoes used in these experiments were three to eight day-old nulliparous females. The mosquitoes were starved from sugar solution for six hours.

Volunteers

Male volunteers, aged between 18 and 40 years were educated on aims, benefits and risks of the study and recruited on written informed consent. The use of strictly male volunteers was to prevent potential risk of malaria infection to pregnant female volunteers. All volunteers were highly experienced in performing human-landing catches. During the SFS experiments, volunteers were screened daily for parasitaemia using RDTs and if found positive, excluded from participating any further in the

experiments and treated with artemether-lumefantrine (ALU), first-line drug for treatment of malaria in Tanzania. During the field evaluation, in addition to daily screening, volunteers were provided with mefloquine prophylaxis. The volunteers were instructed not to use any fragranced soap or perfume, tobacco or alcohol 12 hours before the start and throughout the experiments.

Repellents

The repellent tested was donated by SC Johnson & Sons Inc (Racine, WI, USA). Three treatments were tested: 1) a lotion-based formulation containing 15% DEET as the active ingredient, being the test product; 2) 15% DEET diluted in absolute ethanol, being the standard control, and 3) a placebo made of a similar lotion formulation as the test product, but lacking the active ingredient, being the negative control. Technicians were blinded to the repellent application.

Repellent application

To establish the amount of repellent required for application in the SFS experiments, surface area of the lower limbs of three adult male volunteers was determined by first measuring the length from ankle to the knee and the circumference of the ankle and knee using a tape measure. The surface area was then calculated using the formula that expresses the lower limb surface as a trapezium or cylinder:

$$\text{Area} = 0.5(c_a + c_k)D_{ka} \quad (1)$$

where c_a is the circumference of the ankle in cm, c_k circumference of the knee, and D_{ka} is the distance between c_a and c_k .

Three volunteers were initially asked to apply the repellent *ad libitum* (the amount they felt was safe to protect from mosquito bites) to their legs. While applying the repellent, the volunteers wore latex gloves to avoid absorption of repellents into their skin, which would otherwise reduce the net quantity of repellent applied. The product bottles were then weighed using a precision weighing balance (Ohaus Corp, Pine Brook, NJ, USA) after this initial application to determine the amount applied by each volunteer. The average amount of repellent per volunteer was then calculated from these results. The average amount applied per volunteer was determined to be 2 mg per volunteer-leg. The average surface area of a volunteer's leg was 1,041 cm². The amount of DEET applied was 0.002 mg/cm² (2 mg/1,041 cm²). After amount of repellent required for application was determined, the PI (SO) premeasured these amounts in a Petri dish for each volunteer every evening. The volunteers were then asked to wear latex gloves and

apply their respective amounts on their lower limbs every evening before the start of each experiment.

Study design

The SFS experiments used a partially randomized, 3 × 3 unbalanced Latin square design. The three treatments used in these experiments were assigned numbers: 1 (15% DEET lotion), 2 (15% DEET ethanol) and, 3 (placebo lotion). Three volunteers were used in these experiments and were randomly assigned to each of the three treatments using the lottery method. The volunteers were also randomly assigned sitting positions inside the SFS using the lottery method, and moved between the positions in the same order every night. One round of repellent evaluation was made up of three nights of mosquito collections, with each volunteer wearing a different treatment and sitting at a different position on each of these nights. A single set of three volunteers conducted these experiments for six nights (two rounds of repellent evaluation). For logistical reasons, the second set of three volunteers conducted the experiments for three nights (one round of repellent evaluation). Therefore, the mosquitoes were collected for a total of nine nights in the SFS, but with two different sets of volunteers. Data from the three rounds was pooled. The authors are aware that this limitation may have increased data variance because of individual variability in attraction of mosquitoes and efficiency in mosquito collections.

The PI (SO) premeasured the amounts of treatments 15 min prior (17.45), to the start of the experiments and asked the volunteers to apply their respective amounts on their lower limbs while wearing latex gloves. The volunteers had also been asked to put on knee-length shorts and ankle high boots, so as to standardize the area of exposure. The volunteers sat on low stools 10 m equidistant from each other in a triangular formation. A cage holding 100 mosquitoes was placed at the centre of this triangle formation. It was determined from literature that the biting rate in the study area was 62.5 bites/person/night [18]. Therefore, 100 mosquitoes were released in each hour in the SFS containing three volunteers to simulate the high biting pressure of the field setting. It was assumed that only half the number of all mosquitoes released would bite the volunteers. Therefore, each volunteer would have received approximately 67 bites/person/night. The average landing rates/volunteer/hour was also determined. At the top of every hour (18.00 h-22.00) the mosquitoes were released by one of the volunteers. The experiments were conducted from 18.00 because this was the reported time of the start of biting activity of vectors in the study area [19]. In total, four cages containing 100 mosquitoes each were used during each night of the SFS experiment. Each volunteer was given a head torch, which they switched on only when

they felt a mosquito landing on their limb or when scanning the legs every 30 seconds for mosquitoes [20]. The volunteers were also given four paper cups, marked from the first to the fourth hour, and instructed to place the catches for each hour in their respective cups. The paper cups were covered with netting that had a hole at the centre to place the mosquitoes into the paper cups, which were plugged using a cotton wool to prevent mosquitoes from escaping. At the end of the experiment (22.00), the mosquitoes collected in the four paper cups were stored in the freezer at the IHI laboratory until the next morning. At 09.00 the next day, the mosquitoes in each paper cup were counted and recorded for each hour. The mosquitoes were then discarded and the paper cups cleaned ready for the day's experiment.

Field evaluation of topically applied repellents

Field evaluation of repellents was conducted in Mbingu village, described above. The experiments were conducted next to the rice fields and away from human dwellings to avoid potential bias in the number and behaviour of mosquitoes [21].

The field evaluation of repellents was conducted using a partially randomized, 3 × 3 balanced Latin square design, in the same manner as the SFS repellent evaluation described above. All field experiments were conducted at the site identified and described above. Six volunteers, two of whom also performed the SFS evaluations, were recruited for field evaluation of repellents. A first set of three volunteers conducted the repellent evaluation for nine nights, followed by the second set of volunteers who also conducted the experiment for nine nights at the same site. Therefore six volunteers evaluated the repellents for a total of 18 nights in the field as it was hypothesised that there would be greater variability in field data and more replicates would be required. The volunteers sat 20 m equidistant from each other in a triangular formation. They collected mosquitoes from 18.00 to 22.00, and placed them in the different paper cups marked one to four hours. At the end of the collections, the paper cups holding the mosquitoes were placed in a cool box containing a piece of cotton wool impregnated with chloroform, which killed the mosquitoes. The next morning the mosquitoes in each paper cup were counted by the respective volunteer and the numbers recorded. The mosquitoes were sorted into anophelines and culicines and stored in separate Petri dishes that were layered with cotton wool and silica gel to prevent desiccation. The mosquitoes were brought back to the IHI laboratory where the culicines were identified to species level by an experienced entomologist using taxonomic keys [22]. The *Anopheles gambiae* complex was identified to species level using polymerase chain reaction (PCR) [23].

Statistical analysis

Calculation of percentage protection

Data from the SFS and field trials were recorded in a Microsoft Excel spreadsheet (Microsoft Corporation), with columns for the date, name of volunteer, treatment the volunteer was wearing, position the volunteer was sitting and the number of mosquitoes caught during each hour. This data was then exported into STATA 11 (StataCorp LP, College Station, Texas, USA), where the total number of mosquitoes caught when using 15% DEET lotion and 15% DEET in ethanol were compared to the total number of mosquitoes caught when using the placebo lotion for each night regardless of who was using it, and an average was calculated. The reductions in number of mosquitoes in these two treatments (15% DEET lotion and 15% DEET in ethanol) were designated protection and expressed as a percentage, (percentage protection). The formula used to calculate percentage protection is shown below:

$$P = [C-T]/C \times 100 \quad (2)$$

where C is the number of mosquitoes caught when the volunteer was using the placebo lotion and T is number of mosquitoes caught when the volunteer was using either the 15% DEET lotion or 15% DEET ethanol.

These results for each night of collection were then aggregated and the average percentage protection when using either 15% DEET ethanol or 15% DEET ethanol calculated using STATA 11.

Poisson regression analysis

Count data was then fitted into a Poisson model in STATA 11, with a log link function and a random intercept for each row of data to account for over dispersion, so as to determine relative risk of being bitten by a mosquito. A Poisson model was chosen because it is used to model count data over a specified period of time, i.e. the number of mosquito bites occurring in one hour. It is also used to model rare events (mosquito bites), which is what was expected when a volunteer was wearing either 15% DEET lotion or 15% DEET ethanol. A Poisson model also allowed for analysis of repeated measures over time on the same individual, i.e. the number of mosquitoes caught by each individual on each day while wearing a different repellent and sitting at a different position. The number of mosquitoes caught/hour was fitted as the dependent variable, and interaction of repellent with time, individual variability and position fitted as predictors. Day (which also accounted for confounders like temperature, humidity and wind speed), was fitted as a random covariate, and a random intercept, in this case a Unique ID, was fitted into the model to account for over dispersion of the data.

The percentage protection of 15% DEET lotion and 15% DEET ethanol per hour and regression coefficients relative to the placebo (Incidence Rate Ratio, IRR) were determined to assess the decay of repellents through time.

Ethical considerations

The volunteers used in these experiments were recruited on written informed consent. In case of any positive blood slide for malaria parasites, ALU combination therapy, the first-line drug for malaria treatment in Tanzania, was available. The volunteers were also informed of the study objectives and that they were free to withdraw their participation at any time during the experiments. The volunteers were experienced in human landing catch techniques and were issued with loose net jackets to prevent the mosquitoes biting the upper parts of the body. For field experiments, the volunteers were provided with mefloquine prophylaxis to protect them against contracting malaria. Ethical approval was granted by the Ethical Review Boards of Ifakara Health Institute (IHRDC IRB A46), the Tanzanian National Institute of Medical Research (NIMR/HQ/R8a/VOL IX/780), and London School of Hygiene of Tropical Medicine (LSHTM 5174).

Results

Semi-field experiments

Average percentage protection

The average percentage protection of 15% DEET lotion in the SFS as calculated from Equation 2 above was 82.13% (95% CI 75.93-88.82) and 71.29% (95% CI 61.77-82.28) for 15% DEET in ethanol over four hours of mosquito collection.

Poisson regression analysis

The relative risk of being bitten by a mosquito over the four hour test when using 15% DEET lotion compared to placebo lotion was reduced by 91.8% (95% CI 85.73-95.79%, IRR = 0.082 z = -8.23, P <0.0001). When 15% DEET ethanol was compared to the placebo lotion, the relative risk of being bitten by mosquitoes was also reduced by 92.30% (95% CI 85.06-95.45%, IRR = 0.077, z = -8.21, P <0.0001) (Table 1). The relative risk of being bitten increased in hours two and three relative to hour one, although these differences were not significant. There was, however, a significant increase in the risk of being bitten in hour four compared to hour one for both 15% DEET lotion IRR = 3.71 (95% CI 1.78-7.78, z = 3.47, P = 0.001) and 15% DEET ethanol IRR = 3.43 (95% CI 1.60-7.39, z = 3.17, P = 0.002). This is an indication of repellent decay over time. There was location bias, with position 3 having a higher risk of being bitten compared to location one, IRR 2.00 (95% CI 1.51-2.66, z = 4.79, P <0.0001). Position

Table 1 Effect of 15% DEET repellent over time, treatment, position and person on *Anopheles arabiensis* in a four-hour repellent evaluation in the semi-field system at Ifakara Health Institute

Treatments	Hours	Incidence rate ratio (IRR) ¹ [95% CI]	Z-test statistic ²	P-value ³
	1	-	-	-
15% DEET in ethanol	2	1.744 [0.796-3.819]	1.39	0.164
	3	1.223 [0.559-2.675]	0.51	0.613
	4	3.708 [1.767-7.780]	3.47	0.001
15% formulated DEET repellent	1	-	-	-
	2	0.877 [0.359-2.140]	-0.29	0.774
	3	1.674 [0.756-3.709]	1.27	0.204
	4	3.439 [1.601-7.386]	3.17	0.002
Treatments				
Placebo	-	-	-	-
15% DEET in ethanol	-	0.082 [0.045-0.149]	-8.23	<0.0001
15% DEET in lotion format	-	0.077 [0.042-0.142]	-8.21	<0.0001
Position				
1	-	-	-	-
2	-	0.818 [0.587-1.139]	-1.19	0.236
3	-	2.000 [1.506-2.656]	4.79	<0.0001
Person				
1	-	-	-	-
2	-	0.619 [0.441-0.868]	-2.78	0.005
3	-	2.372 [1.796-3.133]	6.08	<0.0001

¹The data for position one, person one and effect of treatments in hour one were used as a reference values for calculating the incidence rate ratios (IRR) for mosquito bites. ²The test statistic z is the ratio of the Coefficient to the Standard error of that respective predictor and is used to test against a two-sided alternative hypothesis that the Coefficient is not equal to zero. ³The probability (P) that a particular z test statistic is different to what has been observed under the null hypothesis.

3 within the SFS was located closest to a nearby restaurant and the mosquitoes were probably more attracted to the light and human cues. There was variability in individual attractiveness to mosquitoes, (Table 1).

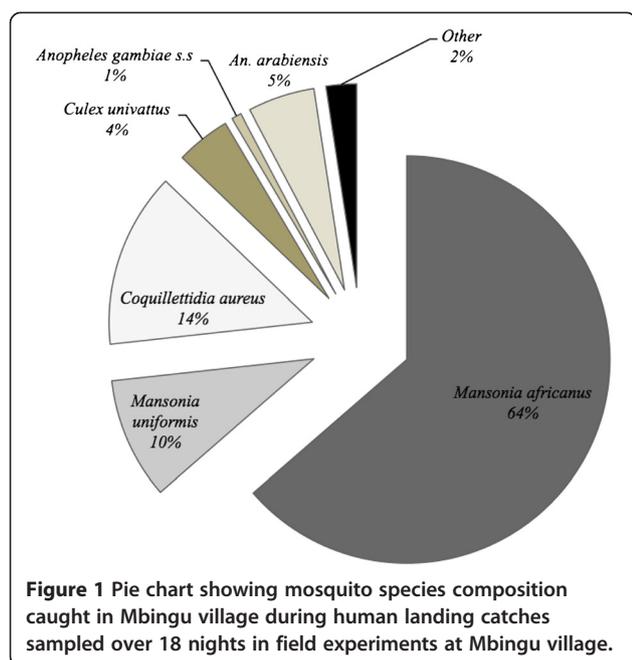
Field trial experiments

Mosquito species composition in the study area

A total of 4,844 mosquitoes were caught in 72 hours over 18 nights. The catch included: 295 (5.4%) *An. gambiae s.l.*, 3,082 (64.6%) *Mansonia africanus*, 467 (9.8%) *Mansonia uniformis*, 673 (14.1%) *Coquillettidia aureus*, 210 (4.4%) *Culex univattus* and 177 (3.7%) other *Culex* species (Figure 1).

Anopheles gambiae s.l. composition in the study area

All the *An. gambiae s.l.* caught were identified to species level by PCR. Out of the 295 successful PCR amplifications,



12.88% (n = 38) were *An. gambiae s.s.*, while 87.12% (n = 257) were *An. arabiensis* (Figure 1).

Average percentage protection

The average percentage protection, of 15% DEET lotion in the field was 85.10% (95% CI 78.97-91.70) and 88.24% (95% CI 84.45-92.20) for DEET ethanol over four hours of mosquito collection, as calculated from Equation 2.

Poisson regression analysis

The relative risk of being bitten by a mosquito over the four hour test when using 15% DEET lotion was reduced by 94.78% (95% CI 91.46-96.81%, IRR = 0.052, z = -11.74, P <0.0001) compared to the placebo lotion and 96.41% (95% CI 93.94-97.88%, IRR = 0.035, z = -12.42, P <0.0001) while using 15% DEET in ethanol (Table 2).

The risk of being bitten in the fourth hour increased three-fold compared to the first hour when using 15% DEET in ethanol IRR = 3.03 (95% CI 1.52-6.01, z = 3.17, P = 0.001). There was, however, no significant increase in the risk of bitten through hours 1 to 4 when using 15% DEET lotion repellent (Table 2). There was lower variability in individual attractiveness to mosquitoes, with only volunteer 2 being significantly more attractive to mosquitoes, IRR = 4.89 (95% CI 3.51-6.82, z = 9.38, P <0.0001). This individual was consistently more attractive in all field experiments. In this field study, the volunteers recruited had differing body mass. There were volunteers who had a larger body mass than this individual but caught fewer mosquitoes when they were compared. Also, even though all team members were highly experienced, there were more experienced field

Table 2 Effect of 15% DEET repellent over time, treatment, position, and person on total number of mosquitoes in a four-hour repellent evaluation in the Mbingu village

Treatments	Hours	Incidence rate ratio ¹ [95% CI]	Z-test statistic ²	P-value ³
	1	-	-	-
15% DEET in lotion format	2	0.839 [0.422-1.667]	-0.50	0.618
	3	1.133 [0.578-2.222]	0.37	0.714
	4	1.699 [0.873-3.307]	1.56	0.118
15% DEET in ethanol	1	-	-	-
	2	0.791 [0.381-1.641]	-0.63	0.529
	3	2.049 [1.027-4.090]	2.04	0.042
	4	3.027 [1.524-6.011]	3.17	0.002
Treatments				
Placebo	-	-	-	-
15% DEET in lotion format	-	0.052 [0.038-0.085]	-11.74	<0.0001
15% DEET in ethanol	-	0.035 [0.021-0.060]	-12.42	<0.0001
Position				
	1	-	-	-
	2	1.091 [0.851-1.400]	0.69	0.498
	3	0.876 [0.684-1.123]	-1.04	0.299
Person				
	1	-	-	-
	2	4.892 [3.511-6.816]	9.38	0.000
	3	1.392 [0.973-1.987]	1.81	0.070
	4	1.065 [0.624-1.820]	0.23	0.815
	5	0.933 [0.54 0-1.611]	-0.25	0.804
	6	1.377 [0.808-2.347]	1.18	0.239

¹The data for position one, person one and effect of treatments in hour one were used as a reference values for calculating the incidence rate ratios (IRR) for mosquito bites. ²The test statistic z is the ratio of the Coefficient to the Standard error of that respective predictor and is used to test against a two-sided alternative hypothesis that the Coefficient is not equal to zero. ³The probability (P) that a particular z test statistic is different to what has been observed under the null hypothesis.

technicians who did not catch as many mosquitoes as this individual. All volunteers use the same concentration and gram/cm² repellents per body surface area, ruling out the potential bias of one volunteer applying more repellent. Studies have shown variable responses of mosquitoes to singular or constituent host attractive cues. It is therefore likely that, the combination of this volunteers body cues/odours [24], made him more attractive to mosquitoes than the combination of cues that were emitted by the other volunteers.

Anopheles gambiae experiments

Data on *An. gambiae s.l.* from the study area was analysed separately to determine the efficacy of repellents on this species of major medical importance.

Average percentage protection

The average percentage protection of 15% DEET lotion in the field was 93.40% (95% CI 89.21-97.79) and 91.45% (95% CI 85.79-97.47) for 15% DEET in ethanol over four hours of mosquito collection, as calculated from Equation 2.

Poisson regression analysis

The relative risk of being bitten when using 15% DEET lotion was reduced by 82.86% (95% CI 53.26-93.71, IRR = 0.171, $z = -3.45$, $P = 0.001$) when compared to placebo lotion and by 83.43% (95% CI 55.81-93.79, IRR = 0.165, $z = -3.59$, $P < 0.0001$) when using 15% DEET in ethanol over the four hours of the test. There was no significant difference in the average number of *An. gambiae s.l.* caught at the different positions in the field, in each hour or by each treatment in each hour over the four hours of mosquito collections demonstrating consistent protection. There was however a significant difference in the average number of *An. gambiae s.l.* caught by volunteer 2: IRR = 2.66 (95% CI 1.42-4.98, $z = 3.06$, $P = 0.002$) and volunteer 6: IRR 0.26 (95% CI 0.81-0.84, $z = -2.25$, $P = 0.025$) relative to volunteer 1 (Table 3).

Comparison of full field and semi-field system data

Decay of repellent from the Poisson regression equations (Tables 1 and 2) and the linear regression demonstrated that 15% DEET in lotion format decayed at a slower rate than 15% DEET in ethanol in both the SFS and field settings. A linear regression also demonstrated a similar trend with regression coefficients showing a more rapid decay of 15% DEET in ethanol in the SFS and against all mosquitoes in the field, with equal decay of the two formulations against *An. gambiae s.l.* in the field (Table 4). However, the results from the linear regression equations (regression coefficients) should be interpreted with caution as the data were over dispersed even after transformation to a proportion (percentage protection) and Linear regression is a parametric test that assumes equal variance around the mean. The percentage protection provided by 15% DEET lotion and 15% DEET in ethanol was similar in the SFS and field settings and on both occasions both treatments provided greater protection in the field than in the SFS (Figure 2). When the two treatments (15% DEET lotion and 15% DEET ethanol) were compared statistically there was no difference between the two measured in the SFS IRR = 0.904 (95% CI 0.44-2.80, $p = 0.833$) nor the field IRR = 0.621 (95% CI 0.316-1.221, $p = 0.168$).

Discussion

The epidemiology of malaria in sub-Saharan Africa is experiencing a subtle shift. Before the advent of LLINs and indoor residual spraying (IRS), malaria transmission was

Table 3 Effect of 15% DEET repellent over time, treatment, position, and person on *Anopheles arabiensis* in a four-hour repellent evaluation in the Mbingu village

Treatments	Hours	Incidence rate ratio (IRR) ¹ [95% CI]	Z-test statistic ²	P-value ³
	1	-	-	-
15% DEET in lotion format	2	0.403 [0.083-1.956]	-1.13	0.260
	3	0.326 [0.068-1.550]	-1.41	0.159
	4	0.722 [0.185-2.812]	-0.47	0.639
	1	-	-	-
15% DEET in ethanol	2	1.229 [0.343-4.399]	0.32	0.750
	3	1.963 [0.583-6.621]	1.09	0.277
	4	1.370 [0.400-4.693]	0.86	0.500
Treatments				
Placebo	-	-	-	-
15% DEET in lotion format	-	0.171 [0.063-0.467]	-3.45	0.001
15% DEET in ethanol	-	0.165 [0.062-0.441]	-3.59	<0.0001
Position				
	1	-	-	-
	2	0.932 [0.542-1.602]	-0.25	0.800
	3	1.262 [0.750-2.126]	0.88	0.380
Person				
	1	-	-	-
	2	2.660 [1.420-4.979]	3.06	0.002
	3	1.801 [0.924-3.510]	1.73	0.084
	4	0.381 [0.127-1.141]	-1.72	0.085
	5	0.328 [0.106-1.015]	-1.93	0.053
	6	0.262 [0.081-0.841]	-2.25	0.025

¹The data for position one, person one and effect of treatments in hour one were used as a reference values for calculating the incidence rate ratios (IRR) for mosquito bites. ²The test statistic z is the ratio of the Coefficient to the Standard error of that respective predictor and is used to test against a two-sided alternative hypothesis that the Coefficient is not equal to zero. ³The probability (P) that a particular z test statistic is different to what has been observed under the null hypothesis.

mediated indoors and late in the night mainly by *An. gambiae s.s.* This species of the *An. gambiae* complex is known to be predominantly anthropophilic, endophagic and endophilic [25,26]. This characteristic is responsible for the success of LLINs and IRS in controlling *An. gambiae s.s.*, as these tools predominantly target indoor biting and resting malaria vectors. However, *An. arabiensis*, the other dominant vector species of the *An. gambiae* complex [26] exhibits a more plastic behaviour [27]. In areas where the host is predominantly human and found indoors, this vector displays anthropophilic, endophagic and endophilic behaviour, similar to its sibling species, *An. gambiae s.s.* However in areas where the host are

Table 4 Comparison of rate of decay of repellents, percentage protection and log-transformed means of mosquito catches per hour in the semi-field system against *Anopheles arabiensis* and in the field against all mosquito species and *Anopheles arabiensis*

Experiment	Hour	Regression equation	Treatments	GEOMEAN	Percentage protection (CI)*
Semi-field evaluation against <i>An. arabiensis</i>	1			2.69	90.88 (84.25-98.03)
	2	$Y = -0.0765 + 1.0315$	Lotion-based 15% DEET repellent	1.7	91.85 (84.85-99.43)
	3			3.1	82.60 (70.39-96.93)
	4	$R^2 = 0.29138$		4.63	65.97 (52.28-83.24)
	1	$Y = -0.119x + 0.9685$	15% DEET in ethanol	4.65	75.55 (51.79-110.20)
	2			3.63	70.76 (54.63-91.65)
	3	$R^2 = 0.08181$		3.17	82.18 (61.19-110.36)
	4			6.26	58.42 (40.45-84.36)
Field evaluation against all mosquito species	1			4.77	87.39 (76.49-99.83)
	2	$Y = -0.0077x + 0.8921$	Lotion-based 15% DEET repellent	4.03	88.92 (79.15-99.88)
	3			5.44	85.99 (76.30-96.90)
	4	$R^2 = 0.00174$		8.03	83.98 (73.78-94.19)
	1	$Y = -0.0427x + 1.0009$	15% DEET in ethanol	4.22	91.98 (84.14-100.55)
	2			5.94	95.11 (91.02-99.37)
	3	$R^2 = 0.11871$		10.89	87.87 (83.08-92.95)
	4			13.5	79.03 (69.14-90.33)
Field evaluation against <i>An. arabiensis</i>	1			1.22	92.58 (83.18-103.05)
	2	$Y = 0.0311x + 0.7904$	Lotion-based 15% DEET repellent	1.25	100.00 (100.00-100.00)
	3			1	92.60 (84.30-101.72)
	4	0.06763		1.64	88.02 (76.15-101.75)
	1	$Y = 0.0208 + 0.6235$	15% DEET in ethanol	0.72	95.20 (87.33-103.78)
	2			0.94	94.93 (87.85-102.57)
	3	$R^2 = 0.045263$		1.5	82.26 (61.18-110.61)
	4			1.17	91.15 (83.82-101.31)

*Some confidence intervals exceed 100% because the ranges were calculated by regression analysis using continuous data. They should therefore be read as 100% efficacy.

found outdoors and are non-human, *An. arabiensis* readily shifts to exophagic, exophilic and zoophagic behaviour [25]. Therefore, extensive and long-term employment of LLINs and IRS is likely to significantly diminish and in some situations completely eliminate the populations of *An. gambiae s.s.*, thereby selecting for the highly adaptable *An. arabiensis* that predominantly bites early in the evening and outdoors [27]. As a result, even though LLINs and IRS will decrease malaria transmission as a whole, there will be a substantial proportion of residual transmission occurring outdoors and in the early evenings that these intradomestic tools cannot tackle [27].

Consequently, there is a need to develop novel tools or methods that can tackle this residual transmission. Repellents, both topical and spatial, provide a promising solution for controlling outdoor transmission [28-30].

However before topical repellents are employed in the community, their performance needs to be correctly and accurately measured under user conditions. It is, therefore, essential to develop a robust methodology for testing repellent efficacy that is representative of conditions under which the repellents are used (the community), but does not expose individuals conducting these experiments to potential malaria vectors [1,2]. It was hypothesized that locating the SFS in regions representative of ambient conditions for the targeted disease vector and testing repellents on humans against these vectors is likely to yield results that correlate well with field tests. Therefore, to qualify the effect of these treatments in these two settings, data for *An. arabiensis* in the SFS was analyzed against data of *An. gambiae s.l.* in the field experiments (as > 80% of this species complex was found to be *An. arabiensis*).

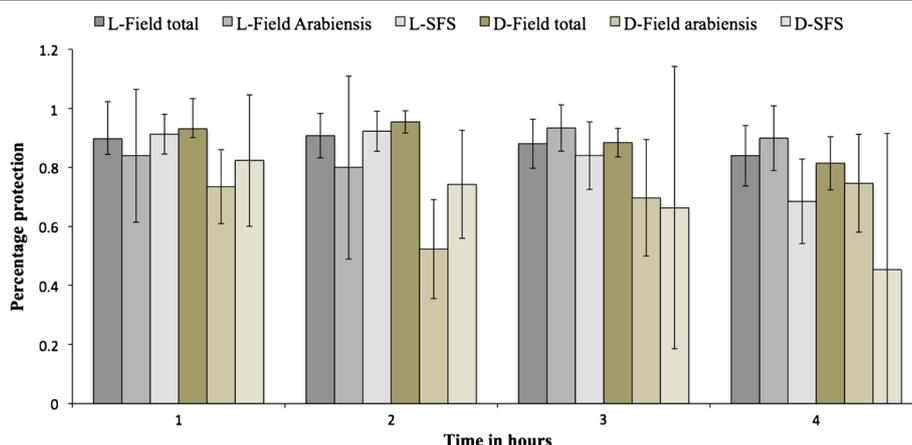


Figure 2 Comparison of percentage protection of 15% DEET lotion repellent and 15% DEET ethanol against *Anopheles arabiensis* in the semi-field system, all mosquito species in the field and *Anopheles arabiensis* in the field after four hours of mosquito collection.
 L-Field total is 15% DEET lotion tested against all mosquito species in the field. L-Field *Arabiensis* is 15% DEET lotion against *An. arabiensis* in the field. L-SFS is 15% DEET lotion against *An. arabiensis* in the semi-field system. D-Field total is 15% DEET in ethanol tested against all mosquito species in the field. D-Field *Arabiensis* is 15% DEET in ethanol against *An. arabiensis* in the field. D-SFS is 15% DEET in ethanol against *An. arabiensis* semi-field system.

The findings demonstrated that 15% DEET lotion protected against 82.13% (95% CI 75.93-88.82) of the bites in the SFS compared to 93.40% (95% CI 89.21-97.79) protection against bites in the field, while 15% DEET in ethanol protected against 71.29% (95% CI 61.77-82.28) bites in the SFS compared 91.45% (95% CI 85.79-97.47) bites in the field against *An. gambiae s.l.* These results demonstrate that both 15% DEET lotion and 15% DEET repellent were more efficacious in the field than in the SFS. A plausible explanation for this might be the high biting pressure observed in the SFS compared to the field. Mosquitoes were exposed to fewer hosts than they normally would in the field and their numbers were continuously increased from 100 mosquitoes in the first hour to 400 mosquitoes in the fourth hour (Figure 2, Tables 5 and 6). By simulating high biting pressure that increased over time as is seen in the field due to the circadian rhythm of the local malaria vectors [19], the authors ensured that the repellent worked extremely well against the predominant malaria vector species before going to the more dangerous field setting. It is known that repellents have varying effects on the other mosquito species present in the field [6,31]. As a result, the effect of the repellent in the field might be over or underestimated depending on the other species present in the field. It is, therefore, prudent, that before the effect of a repellent is established, it should be tested against different mosquito species to assess its efficacy. These data showed that DEET efficacy against one Anopheline species only in the SFS was similar to that for a range of non-anophelines in the full field although this needs to be

validated for other repellent classes, as not all repellents are broad-spectrum.

It is often assumed that formulated repellents provide longer protection against arthropod bites, especially those that have a high vapour pressure. However, findings from this study demonstrate that this may not always be true, and that different formulations of

Table 5 Mean landing rates (MLR) of *An. arabiensis*/volunteer/hour in a four hour repellent evaluation in the Semi-field system at the Ifakara Health Institute

	Volunteer 1 median (IQR)	Volunteer 2 median (IQR)	Volunteer 3 median (IQR)
Placebo			
Hour 1	17 (6–20)	22 (11–27)	41 (19–46)
Hour 2	16 (13–19)	18 (8–18)	17 (16–43)
Hour 3	14 (10–24)	24 (6–29)	37 (18–56)
Hour 4	14 (11–30)	16 (8–20)	28 (12–36)
15% DEET in ethanol			
Hour 1	0	0	12 (1–13)
Hour 2	1 (0–3)	1 (0–5)	8 (7–10)
Hour 3	1 (0–1)	0 (0–1)	9 (6–19)
Hour 4	4 (1–10)	4 (0–4)	19 (7–18)
15% DEET in lotion formulation			
Hour 1	2 (0–4)	0 (0–1)	4 (2–6)
Hour 2	1 (1–5)	2 (0–2)	1 (1–5)
Hour 3	3 (2–15)	2 (0–2)	3 (2–4)
Hour 4	3 (2–17)	3 (2–5)	8 (4–10)

Table 6 Mean landing rates of *An. gambiae* s.l/volunteer/hour in a four hour repellent evaluation in Mbingu village

	Volunteer 1 median (IQR)	Volunteer 2 median (IQR)	Volunteer 3 median (IQR)	Volunteer 4 median (IQR)	Volunteer 5 median (IQR)	Volunteer 6 median (IQR)
Placebo						
Hour 1	10 (2–10)	2 (0–3)	4 (1–5)	0 (0–2)	0 (0–6)	(0)
Hour 2	2 (1–7)	4 (2–4)	3 (1–4)	2 (0–5)	1 (0–3)	1 (0–3)
Hour 3	4 (1–22)	3 (0–6)	10 (1–13)	0 (0–3)	0 (0–4)	0 (0–4)
Hour 4	4 (0–6)	3 (1–7)	11 (3–12)	0 (0–8)	2 (0–3)	0 (0–5)
15% DEET in ethanol						
Hour 1	0 (0–0)	2 (1–9)	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)
Hour 2	0 (0–1)	1 (0–7)	2 (0–6)	0 (0–0)	0 (0–0)	0 (0–0)
Hour 3	0 (0–3)	4 (1–8)	2 (0–4)	0 (0–5)	0 (0–0)	0 (0–0)
Hour 4	0 (0–0)	4 (1–5)	3 (1–6)	0 (0–0)	0 (0–0)	0 (0–1)
15% DEET in lotion						
Hour 1	0 (0–0)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–2)	1 (0–1)
Hour 2	0 (0–0)	0 (0–2)	0 (0–0)	1 (0–1)	0 (0–0)	0 (0–0)
Hour 3	0 (0–1)	1 (0–1)	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–0)
Hour 4	0 (0–0)	2 (0–3)	0 (0–2)	0 (0–0)	0 (0–0)	0 (0–0)

repellents containing the same amount of active ingredient (AI) provide relatively similar efficacy against arthropod bites. These findings are similar to a study carried out to test the efficacy of different formulations of repellents against ticks [32,33].

This is the first study known to have compared the efficacy of topical repellent in both the SFS and field and to determine a correlation between these two settings. However, the current study did suffer from some shortcomings, and an attempt to outline a rationale procedure for conducting future studies incorporating the lessons learnt from this study is suggested below.

A fully randomized, balanced Latin square design should be employed, so that each volunteer tests each of the repellents in all positions available in the SFS. Each volunteer should test each treatment for an equal number of days in each position. The treatments and positions should be randomly assigned to the volunteers and the movement through these positions should be also be randomized. The exact number volunteers testing the repellents should be established, and this number used to calculate the average repellent dose to be applied per individual/surface area. This is to avoid under or overestimating the repellent dose required per person in a case where fewer or more individuals are used to establish the amount of repellent required than those actually testing the repellents. Each group of volunteers testing the repellents should perform an equal number of replicates so that the results are not confounded by individual variability in attractiveness of mosquitoes, a bias that is minimised when all volunteers have equal number of replicates. All

repellent application should be done by an individual wearing gloves, either by the volunteers themselves or an assistant, to prevent repellent absorption into the skin, thereby reducing net amount of repellent being applied. The local dominant vector species, the biting rate per night and time of biting should be established and the number of mosquitoes representative of the biting rate used in the study. The experiments should also be started at the beginning of peak biting activity of the dominant vector in the local area, to avoid interfering with the circadian rhythm. Varying the biting pressure and peak biting times may vary the results of the SFS.

Using a new model of repellent efficacy as a function of user compliance and malaria intensity developed by SJM and Briet (personal communication), the predicted reduction in malaria provided by the repellent in this scenario would be 44%, assuming 80% repellent efficacy and 80% compliance among users with a sporozoite index of 0.005637 (Okumu, personal communication), a transmission season of 200 days per year and biting pressure of 32 bites per night from the major malaria vector *An. arabiensis* [34].

Conclusion

The findings of this study support the hypothesis that repellent testing conducted in SFS yields similar results to field tests, and could be used in place of field tests, to avoid unnecessary exposure of volunteers to potentially infectious disease vectors, provided repellent efficacy is established against a range of representative mosquito species.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SJM and SO conceived the study; SO, EM, DL, HN, DL, JK, and EM performed data collection; MM identified mosquitoes; SO and SJM performed analysis; SO wrote the manuscript; SJM and MM commented on the manuscript. All authors have agreed to the final version.

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Author details

¹Ifakara Health Institute, Box 74, Bagamoyo, Tanzania. ²Disease Control Department, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK. ³Department of Health Interventions, Swiss Tropical and Public Health Institute, Socinstrasse, 57, CH-4002 Basel, Switzerland. ⁴University of Basel, Petersplatz 1, 4003 Basel, Switzerland.

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Registry

T: +44(0)20 7299 4646

F: +44(0)20 7299 4656

E: registry@lshtm.ac.uk

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Thesis Title	CAN REPELLENTS PREVENT MALARIA IN TANZANIA?

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RESEARCH

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A cluster-randomized controlled trial to assess the effectiveness of using 15% DEET topical repellent with long-lasting insecticidal nets (LLINs) compared to a placebo lotion on malaria transmission

Onyango Sangoro^{1,2*}, Elizabeth Turner³, Emmanuel Simfukwe¹, Jane E Miller⁴ and Sarah J Moore^{1,5,6}

Abstract

Background: Long-lasting insecticidal nets (LLINs) have limited effect on malaria transmitted outside of sleeping hours. Topical repellents have demonstrated reduction in the incidence of malaria transmitted in the early evening. This study assessed whether 15% DEET topical repellent used in combination with LLINs can prevent greater malaria transmission than placebo and LLINs, in rural Tanzania.

Methods: A cluster-randomized, placebo-controlled trial was conducted between July 2009 and August 2010 in a rural Tanzanian village. Sample size calculation determined that 10 clusters of 47 households with five people/household were needed to observe a 24% treatment effect at the two-tailed 5% significance level, with 90% power, assuming a baseline malaria incidence of one case/person/year. Ten clusters each were randomly assigned to repellent and control groups by lottery. A total of 4,426 individuals older than six months were enrolled. All households in the village were provided with an LLIN per sleeping space. Repellent and placebo lotion was replaced monthly. The main outcome was rapid diagnostic test (RDT)-confirmed malaria measured by passive case detection (PCD). Incidence rate ratios were estimated from a Poisson model, with adjustment for potential confounders, determined *a priori*. According-to-protocol approach was used for all primary analyses.

Results: The placebo group comprised 1972.3 person-years with 68.29 (95% C.I. 37.05-99.53) malaria cases/1,000 person-years. The repellent group comprised 1,952.8 person-years with 60.45 (95% C.I. 48.30-72.60) cases/1,000 person-years, demonstrating a non-significant 11.44% reduction in malaria incidence rate in this group, (Wilcoxon rank sum $z = 0.529$, $p = 0.596$). Principal components analysis (PCA) of the socio-economic status (SES) of the two groups demonstrated that the control group had a higher SES (Pearson's chi square = 13.38, $p = 0.004$).

Conclusions: Lack of an intervention effect was likely a result of lack of statistical power, poor capture of malaria events or bias caused by imbalance in the SES of the two groups. Low malaria transmission during the study period could have masked the intervention effect and a larger study size was needed to increase discriminatory power. Alternatively, topical repellents may have no impact on malaria transmission in this scenario. Design and implementation of repellent intervention studies is discussed.

Trial registration: The trial was registered ISRCTN92202008 - <http://www.controlled-trials.com/ISRCTN92202008>

* Correspondence: psangoro@ihi.or.tz

¹Ifakara Health Institute, Box 74, Bagamoyo, Tanzania

²Disease Control Department, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

Full list of author information is available at the end of the article

Background

In the past decade, considerable financial and political resources have been mobilized for malaria control [1]. This has in turn led to extensive coverage and use of existing control tools, like long-lasting insecticidal nets LLINs and indoor-residual spraying (IRS) [1]. Implementation of these highly effective vector control tools has resulted in substantial decrease in malaria transmission, morbidity and mortality [2-4]. Despite both extensive coverage and use, the sole use of these tools have not and will not be able to eliminate malaria in all malaria endemic regions [5]. Because LLINs and IRS target mainly indoor biting and indoor resting vectors their implementation may select for outdoor resting and biting vector populations that often become dominant, so that even though there is a diminished malaria transmission as a result of extensive LLINs and IRS use, there is likely to be a larger proportion of this residual transmission occurring outdoors compared to indoors [6].

Increased urbanization and rural electrification programmes have also had an impact on malaria transmission dynamics. As a result of this, individuals stay up later in the evenings than they usually would in a situation where electricity was not available [7], and are, therefore, exposed to potentially infective mosquito bites for longer.

With the renewed push for malaria elimination [8], it is evident that new tools need to be developed to augment existing vector control tools to achieve this goal. Topical repellents provide excellent personal protection [9] and could potentially be used to complement LLINs for additional protection from residual transmission [5]. Several studies demonstrated that topical repellents offer additional protection from malaria transmission either when used alone, or in combination with LLINs, in areas with high early evening and outdoor malaria transmission [10-12].

This study assessed the potential additional benefit of using topical repellents in combination with LLINs compared to using only LLINs on early evening malaria transmission in a rural community in Kilombero valley, south-west Tanzania.

This community mainly relies on subsistence farming of rice, which provides for a large breeding site for both malaria vectors and nuisance biting mosquitoes [13]. It is customary that the community in the study area cook outdoors in the early evenings, a situation that is likely to expose them to mosquito bites and potential malaria transmission. Rural development is also rapidly taking place in this study area. As a result, many members of the community usually gather in the early evening and stay late into the night at local entertainment spots that are springing up in the study area owing to rural electrification programmes, thereby increasing the potential of malaria transmission at these times. A recent report estimates a malaria incidence rate of 0.67 cases/person/year confirmed by rapid diagnostic test (RDT) from passive case detection at a local clinic

between December 2012 and July 2013 (Jabari Mohammed Namamba, pers. comm.).

In the past two decades, extensive malaria intervention programmes have taken place in this area, and it is therefore expected that the community be highly sensitized on malaria transmission and control methods [14-17]. There is high LLIN use in the study area [18]. Repellent awareness and knowledge as assessed using a Knowledge, Attitude and Practice (KAP) baseline questionnaire at the inception of the clinical trial determined that this community did not use topical repellents as a mosquito control tool. Awareness and availability were reported as the major reasons for not using topical repellents [Sangoro O, Sarah M, Ann HK, Sarah M: **Feasibility of repellent use in a context of increasing outdoor transmission: A Qualitative study in rural Tanzania, submitted to Malaria Journal for publication**].

The major malaria vector in the study area is *Anopheles arabiensis* [19], which has been shown to exhibit elastic feeding behaviour depending on the availability and location of the host [6] and is known to exhibit early evening biting [20]. The dominance of this vector in this area is also likely to be the result of extensive LLIN use in the study area [21,22].

A field study conducted in the study area to determine the efficacy of this repellent (15% DEET) against *An. arabiensis* demonstrated >80% protection from bites over four hours of mosquito collection [19]. Therefore, 15% DEET was considered appropriate to provide protection against early evening biting.

This study area was chosen because there are no studies that have been conducted to assess the additional benefits of topical repellents to LLINs in malaria control in East Africa, although this technology has been shown to work elsewhere in sub-Saharan Africa [23,24]. Also the vectors present in the area, *An. arabiensis*, exhibit early evening biting [20], a trait that made the use of repellents in the early evening ideal in this area. Therefore, even though extensive employment of current control tools will lower malaria transmission in this area, it is likely that residual transmission will continue occur at times when the effectiveness of these tools is diminished, like outdoors in the early evenings and mornings, [6] and will require supplementary tools that target this scenario.

Therefore, it was hypothesized that combined use of LLINs and topical repellents in this community would have a greater impact on malaria transmission in the early evening compared to sole use of LLINs.

Methods

Study area

The study was carried out in Mbingu village, Ulanga district, situated 55kms west of Ifakara town at 8.195°S and 36.259°E. At the time of the study inception, (July 2009),

the village was estimated to have 7,609 inhabitants [25]. There is moderate malaria transmission in the study area, with peak transmission occurring in the months of May and June after the long rains. The village experiences an annual rainfall of approximately 1,200-1,800 mm and an annual temperature range of between 20°C and 32.6°C. The village borders an extensive field cleared for rice irrigation, which provides an ideal breeding site for malaria vectors [13].

Sample size rationale

The only available data from the study area were community reported fever incidence rate estimates of 3.2 cases/person/year for children under the age of five years [26]. Assuming fever rates in children under five years are higher than the rest of the population, and that not all fevers reported are caused by malaria, a rate of one malaria case/person/year was used to calculate the sample size needed for this study. Available reports also indicated that 30% of mosquito bites occurs in the early evening [20]. Therefore, assuming that mosquitoes have an equal probability of carrying sporozoites regardless of time of night, it was assumed there was a potential 30% malaria transmission occurring in the early evenings. Expecting that repellents would reduce 80% of this potential 30% early evening transmission, as observed from the field study [19], it was reasoned that repellents would reduce the overall transmission of malaria from one case/person/year to 0.76 cases/person/year. Using the methods of Hayes *et al.* [27] for sample size calculation for cluster randomized trials, it was estimated that to observe this treatment effect (24%), with 90% power at the two-tailed 5% significance level, 10 clusters of 47 households with five members each was required per treatment group. A coefficient of variation (k) of 0.20 was used based on published recommendations as the inter-cluster variation could not be estimated [28].

Household recruitment

Households were recruited into the study in two phases. In phase one, the study investigators and field team visited the study village for reconnaissance and introduction to the community leaders and members in December 2008. A week later, the study team returned to the study village and aided by community leaders, identified the centre of the village. Here, the field team spun a ballpoint pen and visited all the households that the writing end of the pen pointed to with the intention of recruiting all consenting households into the study. After all households in this direction had been exhausted, the field team went back to the village centre and spun the pen to choose the next direction in which to visit the households. If the pen pointed in the direction where the households were already visited, then, the pen was spun again until a new direction was identified. This progression was repeated until approximately,

1,000 households had been visited and recruited. The village had 2,000 households [25] and, therefore, by visiting and potentially enrolling at least 50% of the households, the study team were confident that they had captured a representative sample of households in the study area.

Enrolment of households into the study

During the household recruitment visits, each household head was informed of the purpose of the visit. They were educated on the objectives, risks and benefits of the study to their household and the community. They were encouraged to ask questions and after all their concerns had been addressed, they were asked if they were willing to participate in the study. If willing, each household head was asked to sign a written informed consent form, confirming their participation and that of all household members. As data was being collected at the household level, only the household head was asked for informed consent. It was assumed that once that household head gave consent then all household members would likely comply with repellent use following instructions of the household head as the authority in each household. A structured questionnaire on the socio-economic status (SES) of the household and knowledge, attitude and practice (KAP) in relation to malaria and repellents was then administered [Sangoro O, Sarah M, Ann HK, Sarah M: **Feasibility of repellent use in a context of increasing outdoor transmission: A Qualitative study in rural Tanzania, submitted to Malaria Journal for publication**]. The GPS coordinate of the household enrolled was then recorded using a handheld GPS receiver (Garmin eTrex Legend® H). These coordinates were then plotted using Arc GIS software (Arc GIS 9.0, ESRI, UK), to generate a map of all the households enrolled in the study area.

Second phase of household recruitment, household enrolment and cluster generation

In phase two, the map generated during the first phase of recruitment was used to delineate 20 clusters of households each while ensuring a buffer zone of 200 metres between clusters to prevent diversion of mosquitoes from the intervention group to the control group. As a result of creation of this buffer area, some households that had been recruited in the first phase fell within this 200 metre buffer area. These households were excluded from the study during this second phase of recruitment. Therefore, even though about 1,000 households were recruited in the first phase, more households needed to be recruited in the second phase as a result of loss of households within the buffer area. These households were excluded because they would have potentially confounded the outcome of the study in case of diversion of mosquitoes. All households within the buffer area were issued with an LLIN per sleeping space to protect them from potentially greater than

normal bites from diverted mosquitoes. In practice, the second phase of recruitment proceeded as follows: The field team visited the 20 clusters, using the household considered to be at the centre of these clusters (identified from the Arc GIS map), as the starting point. The household head of the central household in the cluster was informed of the purpose of the visit. If the household had been enrolled during the first phase of household recruitment, then the field team issued an LLIN for every sleeping space, stapled a unique identifier number on the door frame and moved to the next nearest household. If the households had not been enrolled, the household head was informed of the objectives, risks and benefits of the study, enrolled on written informed consent, provided with a unique household identifier and LLINs for each sleeping space, and a SES and KAP questionnaire administered. This progression was repeated until 47 households close together were enrolled to form a single cluster. All 47 households in each of the 20 clusters were enrolled in this manner. The newly enrolled households that did not appear on the map generated in the first phase of recruitment were plotted and the map updated to produce the final map of households recruited into the study (Figure 1).

Clusters were used as the unit of randomization for three reasons: 1) since the intervention would be applied to a community, if proven to be effective, 2) to limit contamination of treatments between households, and 3) to avoid diversion of mosquitoes from individuals who used repellents to those who did not use repellent within the same household or from households using repellents to households that used the placebo, thereby putting non-repellent using individuals and households at a potentially higher risk of contracting malaria [29,30].

Eligibility criteria

All households were eligible to be recruited into the trial and no household was excluded on the basis of household structure, asset or livestock ownership. All individuals older than six months of age were eligible to be recruited into the trial. This age cut-off was used because re evaluation of DEET insect repellent [31] estimated the margin of exposure (MOE) in children less than six months to be less than 100. Margin of exposure is defined as the ratio of dose of DEET used daily to the no observed effect level dose recommended by regulation agencies, which usually consider doses, which result in MOEs of less than 100, unacceptable. Based on this risk assessment, use of DEET was not recommended for children under six months [32].

Randomization of clusters to treatments

All the 20 clusters in the map (Figure 1) were assigned numbers 1 to 20, starting from the left hand side to the right. The cluster numbers were then written down on small pieces of paper, which were placed in a bowl. The principal investigator (PI) and project leader (PL) then drew the pieces of paper from the bowl one at a time. Two three digit numbers (258 and 305) were used to classify clusters into two groups. The first cluster number to be drawn was assigned treatment 258 and the second cluster number assigned treatment 305. This progression was repeated until all the clusters had been assigned to one of the two groups.

Blinding

The repellent and placebo lotion smelt and felt the same and were placed in identical tubes, distinguishable only by the two three-digit numbers known only to the independent

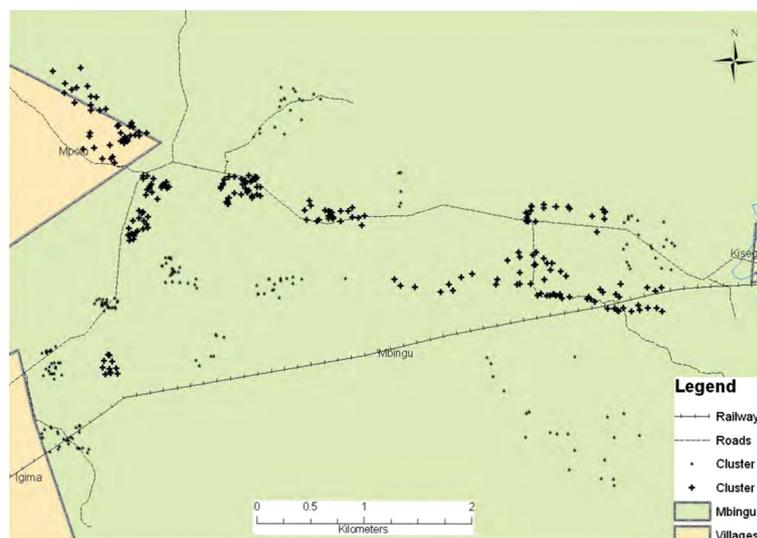


Figure 1 Map of households recruited into the trial in the study village.

code keeper (SC Johnson and Sons). However, the PI and PL had previously conducted efficacy test of these two treatments [19], and could identify the repellent and placebo from the results of this study. Therefore, it was only the field team, study statistician and study participants who were blinded in this study. Blinding was broken after analysis.

Repellent issuance, application and compliance

In June 2009, the field team visited all households enrolled in the study to distribute treatments to study participants. The treatments, (15% DEET and placebo), both formulated as a pourable lotion that is applied by hand, were supplied by SC Johnson, Racine, USA, and packaged in 100 ml plastic tubes. During this visit, the field team informed the household members on how to apply the treatments provided on exposed areas of the body. They also advised the participants not to apply the treatments on open wounds, eyes, mouth and areas with mucous membranes. The repellent lotion was applied at an approximate rate of 0.002 mg DEET/cm², the quantity of repellent that prevented >80% mosquito bites for 4 hours in a controlled environment and in the study area [19]. Even though a repellent with a higher concentration would have provided greater protection, the Tanzania National Institute of Medical Research ethical approval board did not allow the use of a repellent that had more than 15% DEET due to safety concerns, despite the initial request of the PI to use 30% DEET and submission of detailed experimental justification and dossier of safety data justifying the use of a higher concentration.

The participants were issued measuring caps, with amounts of repellent required for adults (7mls) and children below 12 years (3mls) marked on the cap. Each tube held 100mls of repellent. Therefore, two tubes were considered enough to last an adult one month, i.e. if they applied the recommended dosage of 7 mls per day, while one tube was enough to last a child < 12 years for one month, if they used 3mls per day. Children > 12 years were advised to use up to 7mls a day, and were therefore issued with 2 tubes for the month. All the tubes issued per cluster and households were identical, and it is possible that the household members shared a single tube of repellent until it ran out. As all households member were issued with enough treatment to last them month, either 15% DEET repellent lotion or placebo, and dosages for adults and children had been marked out, it was assumed that sharing of repellents within the household would have no effect on the outcome as long as there was daily compliance to the recommended dose by the participants. The amounts recommended were adjusted to accommodate for individuals with greater than average body mass as it was determined from semi-field and field experiments that an average sized volunteer required 6 mls [19]. This amount was, therefore, adjusted upwards

by an extra millilitre. The community members were instructed to apply the repellent at dusk (1800 hrs) and to reapply it if they felt any mosquito bites or remained active for more than four hours after sunset.

Compliance to lotion use (both repellent and placebo) was assessed by the field team visiting the enrolled households at the beginning of each subsequent month (monthly monitoring surveys) to issue new tubes of repellent and placebo lotion. Therefore compliance was assessed on a monthly basis using a short structured questionnaire, where the household head or an adult household member, was asked if all household members had used the repellents and reasons for non-compliance where relevant. However, as self-reported data are unreliable, the number of repellent/placebo tubes issued every month was also recorded as a secondary measure of compliance, to determine if there was a difference in the number of tubes issued in each month per treatment group. Data on use of LLINs the previous night, malaria infection, recalled febrile illness and visit to the health centre during that month was also collected. If, during these monthly monitoring surveys, the household head or any other adult household member was not available to answer the questionnaire on compliance, the field team visited that particular household daily for seven consecutive days. If still no household member able to take the monitoring survey was available during these repeated visits, then that household, and all its members, was excluded from the calculation of person-time for that month.

In addition to the compliance, malaria and recalled febrile illness data collected during each month of the study period, an after study questionnaire was administered at the close of the study to assess the participants' knowledge, attitudes and practice in relation to repellents. These results are reported elsewhere [Sangoro O, Sarah M, Ann HK, Sarah M: **Feasibility of repellent use in a context of increasing outdoor transmission: A Qualitative study in rural Tanzania, submitted to Malaria Journal for publication**].

Clinical data collection

A single government health facility in the study area was recruited into the study. At this facility, health services were provided for free by the project if the participants showed their project identification card with a household unique identification number on it. Community members that were not enrolled into the study were issued with a different kind of identification card to also allow them free consultation and treatment at the recruited health facility. This was done to discourage community members attending the health facility under the guise of being a study participant and, therefore, contaminating the study by recording malaria status of community members not enrolled in the study as participants. It

was assumed that since services were provided for free at this facility, it would attract most community members seeking health services. A clinical officer (CO) and a nurse were employed by the project at this health facility. A ledger with the household unique identifier and names of each household member was drawn up and placed at this health facility. When a study participant visited the health facility with febrile illness, the CO checked against their name and household unique ID in the health facility ledger. This way household and health facility data could be reconciled using the household unique identifier. Febrile participants were tested for malaria using rapid diagnostic test (RDT) (ICT Malaria cassette tests HRPII/pf test kit). A proportion of participants also had diagnosis by thick film microscopy to confirm the accuracy of the RDTs for diagnosis under field conditions. The result of the RDT and the date of diagnosis were marked against the Household ID on the health facility ledger. Those found positive for malaria parasites were given artemether-lumefantrine (ALu), the first-line drug for treatment of malaria in Tanzania. Only participants that were RDT or slide positive for malaria parasites were treated. This was to avoid treating non-malaria patients with ALu, which might have affected malaria incidence rate in the village. The RDT's were labelled with the patient's unique identifier, date and status (+ve or -ve) and stored for verification. These were later checked against the clinical trial database to ensure that no cases had been incorrectly entered into the database by the clinic staff.

Data management

Data from the structured questionnaires on SES of households and KAP in relation to malaria and repellents administered at baseline; follow-up data on compliance and recalled febrile illness administered throughout the study period; and the after study KAP survey, were double entered into a computer using an Epi-Info™ template with a drop down lists of values that corresponded to the format of the questionnaires. Data was then exported to Microsoft Access 2008 (Microsoft Corporation), to check for lack/excesses of data, inconsistencies and outliers. All data from the above mentioned questionnaires were linked using the household unique identifier. The household unique identifier was made up of the household number, cluster number and treatment number.

Statistical analysis

Data was collected and presented at household and cluster level as the study aimed at assessing the effectiveness of the repellents at the community level. Individual level data was not collected.

Socio-economic status (SES)

All data cleaning and analysis was performed using STATA 11.2 software (StataCorp LP, College Station, Texas, USA). Baseline household-level socio-economic indicators were collected using a structured questionnaire. All variables representing asset ownership, household construction materials, source of fuel and light and the education level of the household head were examined individually before being combined using principal component analysis (PCA) to generate the socio-economic index of each household, [33], and are presented in here: (Additional file 1: Stata output showing Eigen scores of each variable used in calculation of socio economic status of households). The households were grouped into quintiles of the socio-economic index generated and ranked from the poorest to the least poor. This data was cross tabulated with treatment group using Pearson's chi-square (χ^2) to assess whether there was a significant difference in the socio-economic status of the households in the two treatment groups (not accounting for the clustered design due to the exploratory nature of this analysis).

The number of treatment tubes issued was analysed by linear regression against month, treatment and an interaction of month and treatment to determine if there was a significant difference in the number of tubes issued in each month and per treatment group.

Clinical data

Clinical data was adjusted for covariates identified *a priori* to be confounders and analysed using the according-to-protocol approach, where person-time at risk was excluded when a participant reported or was observed to be non-compliant to the lotion (placebo or repellent) and for those with malaria for three weeks after they were diagnosed. The total number of cases in each treatment group was divided by the sum of person years at risk to give the incidence rates in person years at risk. Rate ratio and rate differences were then estimated.

For comparison, a secondary analysis using the intention-to-treat approach, where malaria incidence rates in the clusters were compared using all person-time at risk regardless of whether they complied with the study protocol but also adjusted for covariates identified *a priori* as confounders. Such an approach would be expected to underestimate the treatment effect. It was not possible to effectively blind the PI and PL as they had carried out both the semi field and field efficacy evaluations of these treatments [19] and could identify the intervention and placebo. The clinical data was, therefore, re-blinded by an independent statistician (ET), who was not aware of the intervention and placebo codes.

Person-time at risk estimation for according-to-protocol analysis

The study was conducted for 14 months from July 2009 to August 2010. To calculate the person-time at risk, a closed cohort was assumed, so that the number of household members above six months recorded at baseline for each household was assumed to be constant throughout the study period. Monitoring surveys were conducted for each month of the study to establish compliance.

Person time at risk of each household was estimated according to one of the following three possible scenarios:

1. In a case where all individuals were susceptible to malaria infection and complied with the study protocol by applying the treatment issued on a nightly basis, each individual in the household was assumed to contribute one-person month at risk to the study.
2. In a case where the household head or an able household member was not available to take the monthly monitoring surveys, it was assumed that all members of that household did not comply with lotion (repellent or placebo) use for that month and one-person month at risk for each member of that household was excluded from the person time at risk of the study.
3. In a case where a household member contracted malaria, that individual was excluded from calculation of person time at risk for three weeks.

Person-time for all household members was calculated according to the appropriate scenario above.

Malaria incidence rates and regression analysis of the intervention effect

Using data on the total number of confirmed malaria cases and person-time for each household, we used a two-stage approach to estimate intervention effects (recommended by Hayes *et al.* for studies with fewer than 15 clusters/group) [27]. In the first stage, cluster-specific incidence rates were calculated using random effects Poisson regression modelling with adjustment for confounding variables. Specifically, the outcome of total number of confirmed cases of malaria/household was regressed on the set of confounding variables (age categories of the household, education of the household head, and quintile of SES), with an offset for person-time at risk per household and a random intercept for cluster to account for the clustered study design. As per Hayes *et al.*, treatment was not included as a factor in the model. In the second-stage, residuals, calculated from the regression model were aggregated by clusters. The covariate-adjusted treatment effect was then estimated by comparing the residuals in the intervention

relative to the control group using the Wilcoxon rank sum test, because the data were not normal.

Knowledge attitude and practice (KAP) of community members in relation to malaria and repellent

Baseline data on knowledge of malaria and malaria prevention practices and knowledge and practice in relation to repellents were analysed using descriptive statistics in STATA 11.2 to assess whether there was an imbalance between the treatment arms. Data that recorded attitude with regards to repellents, perceived effectiveness and willingness to continue use and pay were also analysed and these results are presented elsewhere [Sangoro O, Sarah M, Ann HK, Sarah M: **Feasibility of repellent use in a context of increasing outdoor transmission: A Qualitative study in rural Tanzania, submitted to Malaria Journal for publication**].

Ethical and safety considerations

During recruitment, the household head was asked for written informed consent for themselves and all household members. If consent was obtained, all members of the household were recruited into the study. Study participants were free to withdraw from the trial at any time. All households in the village were issued with an LLIN for every sleeping space to ensure equity. All individuals from the study village were allowed free consultation, treatment and drugs (ALu) from the village dispensary at project cost. Participant confidentiality was maintained by using generated unique identifiers instead of individual names during analysis.

Participants were educated on correct repellent use and application. Children under 6 months were excluded from the trial. An illustrated label giving instructions in the native language (Swahili) on safe repellent use was provided on each tube. DEET repellent used in this study has undergone extensive toxicological tests and has been endorsed as safe for human use [32]. The concentration of DEET (15%), used in this trial was approved by the Tanzanian Pesticides Research Institute, the Tanzanian Bureau of Standards and is available in Tanzanian shops. Guardians to children < six months were reminded to put their children under an LLIN early to prevent them contracting malaria. A clinical officer (CO) was employed at the village dispensary by the project to perform RDTs and to investigate and treat any adverse effects arising from repellent use.

Ethical approval for the study was obtained from Ifakara Health Institute (IHI) (IHRDC IRB A46), Tanzanian National Institute of Medical Research (NIMR/HQ/R8a/VOL IX/780) and the London School of Hygiene and Tropical Medicine Ethical Review Board (LSHTM ERB 5174). IHI provided study monitoring.

Results

Trial profile and baseline data

The trial profile is summarized in Figure 2. In the intervention group 2,224 individuals were enrolled and 2,202 in the placebo group. Loss-to-follow up was higher in the placebo group: $n = 34$ versus $n = 16$, and no individuals withdrew from the trial. Similar numbers of person-years were analysed: 1952.81 in the intervention group and 1972.38 in the control group of the trial. Baseline household level socio-economic data on education and gender of household head, age-groups of all study participants, household construction material, source of cooking fuel and lighting and asset ownership were examined individually and are presented in Table 1. The gender of the household heads was comparable between the two treatment groups, with 55.33% ($n = 514$) females and 44.67% ($n = 415$) males. Most of the household heads had received some form of formal education, 82.81% ($n = 702$) while only 17.18% ($n = 161$) had no formal education. Of all participants recruited in the study, 17.55% ($n = 771$)

were children under five years of age, 34.37% ($n = 1,510$) were between five to 18 years of age and 48.08% ($n = 2,112$) were above 18 years of age and age-category distribution was similar in the two treatment groups. The predominant source of energy used by the households was wood fire, 89.96% ($n = 883$), while the predominant source of lighting used was the traditional lamp, 93.76% ($n = 871$). Assessment of household construction materials demonstrated that most households in the study area had floors made from mud, 82.78% ($n = 769$), while tin and thatch were used equally as roofing materials, 49.35% ($n = 457$). Also, most households in the study area had walls made from bricks, 79.87% ($n = 742$). Socio-economic indices generated from PCA suggested an imbalance between the two treatment groups, with the control group demonstrating a higher SES than the intervention group, (Pearson's $\chi^2 = 17.5519$, $p = 0.002$), (Table 2).

The use of repellents as a mosquito control tool was low in the study area, with only 1% ($n = 6$) of those interviewed reporting to have ever used repellents. Results on

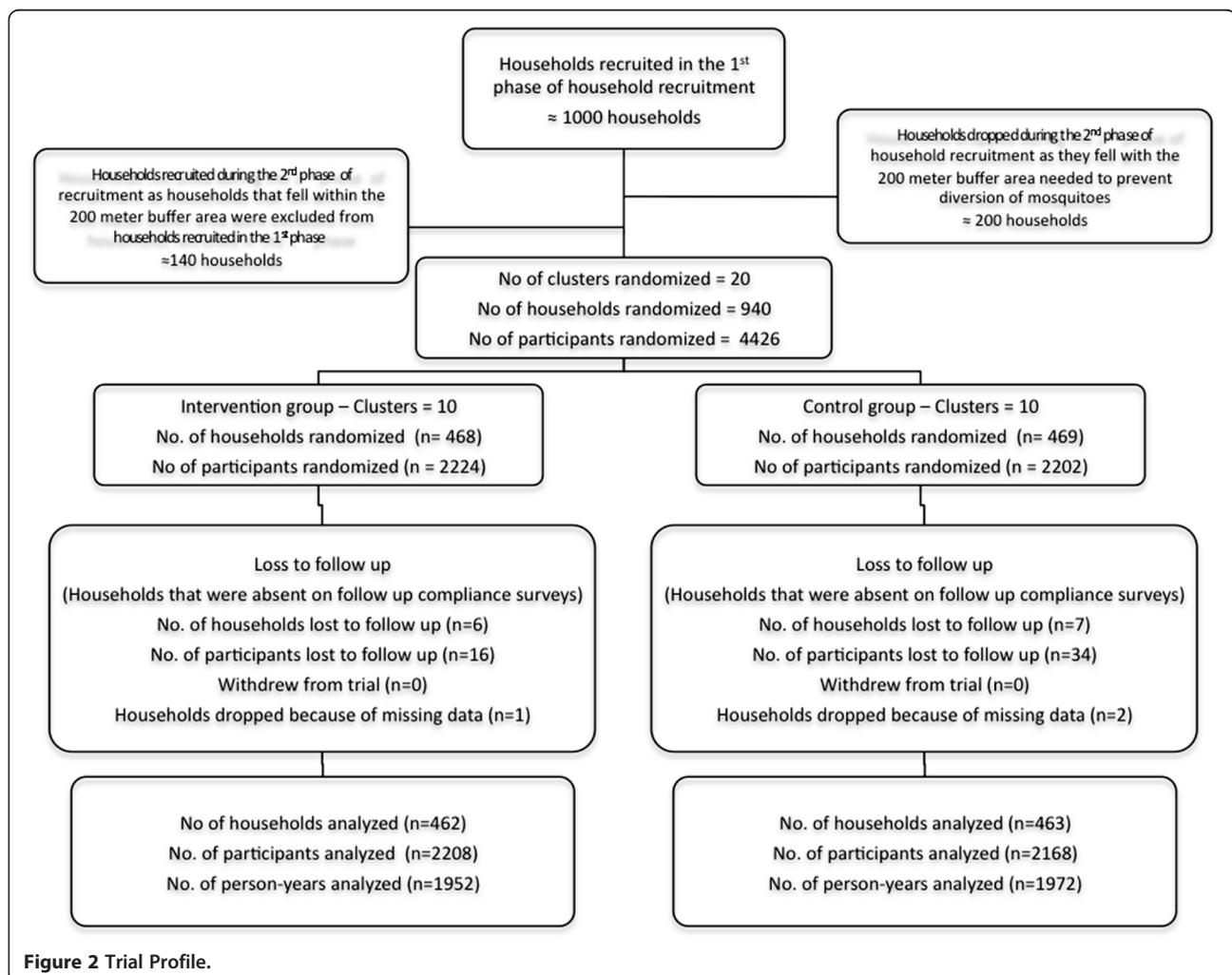


Figure 2 Trial Profile.

Table 1 Baseline household characteristics by treatment group

	Intervention arm n (%)	Control arm n (%)	Totals n (%)
No. of households	469 (50.05)	468 (49.95)	937 (100)
No. of participants	2224 (50.05)	2202 (49.95)	4426 (100)
<i>Gender of household head</i>			
Male	215 (46.24)	200 (43.10)	415 (44.67)
Female	250 (53.76)	264 (56.90)	514 (55.33)
<i>Education of household head</i>			
No education	83 (17.74)	78 (16.63)	161 (17.18)
Educated	385 (82.26)	391 (83.37)	702 (82.82)
<i>Age group distribution of all participant/household</i>			
Under 5's	412 (18.50)	359 (16.57)	771 (17.55)
5-18 years	721 (32.38)	789 (36.43)	1510 (34.37)
Above 18 years	1094 (49.12)	1018 (47.00)	2112 (48.08)
<i>Source of energy</i>			
Wood fire	431 (92.89)	402 (86.83)	833 (89.86)
Other sources	33 (7.11)	61 (13.17)	94 (10.14)
<i>Source of lighting</i>			
Traditional lamp	445 (95.70)	426 (91.81)	871 (93.76)
Other source	20 (4.30)	38 (8.19)	58 (6.24)
<i>Flooring material</i>			
Mud	404 (86.88)	365 (78.66)	769 (82.78)
Cement	61 (13.12)	99 (21.34)	160 (17.22)
<i>Roofing materials</i>			
Thatch	256 (55.41)	201 (43.32)	457 (49.35)
Tin	203 (43.94)	254 (54.74)	457 (49.35)
Other	3 (0.65)	9 (1.94)	12 (1.30)
<i>Wall materials</i>			
Mud	121 (26.08)	66 (14.19)	187 (20.13)
Bricks	343 (73.92)	399 (85.81)	742 (79.87)
<i>Assets ownership</i>			
<i>Motorbike</i>			
Yes	72 (15.48)	52 (11.18)	124 (13.33)
No	393 (84.52)	413 (88.82)	806 (86.67)
<i>Bicycle</i>			
Yes	246 (52.90)	198 (42.58)	513 (55.16)
No	219 (47.10)	267 (57.42)	417 (44.84)
<i>Stove</i>			
Yes	344 (73.98)	314 (67.53)	658 (70.75)
No	121 (26.02)	151 (32.47)	272 (29.25)
<i>Mobile phone</i>			
Yes	197 (42.37)	211 (45.38)	408 (43.87)
No	268 (57.63)	254 (54.62)	522 (56.13)
<i>Radio</i>			
Yes	140 (30.11)	156 (33.55)	296 (31.83)
No	325 (69.89)	309 (66.45)	634 (68.17)

Table 2 Ranking of households using Socio-economic scores generated for PCA analysis by treatment group

	Intervention arm n (%)	Control arm n (%)	Total n (%)	Pearson's Chi2	P value
SES generated from PCA					
Poorest	39 (8.33)	28 (5.97)	67 (7.15)		
Poor	164 (35.04)	121 (25.80)	285 (30.42)	17.5519	0.002
Median	165 (35.26)	174 (37.10)	339 (36.18)		
Less poor	77 (16.45)	107 (22.81)	184 (19.64)		
Least poor	23 (4.91)	39 (8.32)	62 (6.62)		

KAP of repellents are presented in details elsewhere [Sangoro O, Sarah M, Ann HK, Sarah M: **Feasibility of repellent use in a context of increasing outdoor transmission: A Qualitative study in rural Tanzania, submitted to Malaria Journal for publication**].

The average number of tubes issued per household was 6.73 (95% C.I. 6.51 – 6.95) and 6.92 (95% C.I. 6.68 – 7.16) in the intervention and control group respectively and there was no significant difference per treatment group, 1.68 (95% C.I. 0.32 – 84.25, $P = 0.803$) from linear regression analysis. Likewise there was no significant difference on the number of treatment tubes issued per month throughout the study period.

Clinical outcomes

According-to-protocol analysis

When data was analysed as per protocol there was a non-significant difference in cluster and household malaria incidence rates among repellent users and non-users (Table 3). In the cluster-level analysis (data averaged over cluster specific rates), the malaria incidence rates differed by 11.48%; with 68.29 (95% C.I. 37.05-99.53) cases/ 1,000 person-years in the control group and 60.45 (95% C.I. 48.30-72.60) cases/1,000 person-years (95% C.I. 44.55 – 81.73) in intervention group, (Wilcoxon rank sum $z = 0.529$, $p = 0.5967$). For household-level malaria incidence rates (data averaged separately over household specific rates), the incidence rates differed by 28.88%: with 84.54 (95% C.I. 61.04-108.05), cases/1,000 person-years in the control group and 60.12 (95% C.I. 45.08-75.15) cases/1,000 person-years in the intervention group, (Wilcoxon rank sum $z = -1.267$, $p = 0.2051$). These result should however be interpreted with caution as there is still an ongoing debate on whether it is correct to estimate incidence rate ratios using regression models on less than 10 clusters [28]. Cluster aggregated rates were reported because it measured the overall effect of the intervention at the population level [34] and this was the major objective of the study. Age was a significant risk factor with risk decreasing with increase in age. SES did not influence the risk of malaria in the model.

Intention-to-treat analysis

Cluster-level analysis of malaria rates in the two treatment arms demonstrated a non-significant, 14.62% difference in

malaria rates with 53.21 cases/1,000 person-years (95% C.I. 30.98 – 104.16) in the control group and 45.43 cases/1,000 person-years (95% C.I. 36.02 – 59.79) in the intervention group, (Wilcoxon rank sum $z = 0.227$, $p = 0.8206$), (Table 3). Household-level analysis of malaria incidence rates demonstrated a 30.71% difference in malaria incidence rates, with 68.21 cases/1,000 person-years (95% C.I. 49.59 to 86.84) in the control group and 47.26 cases/1,000 person-years (95% C.I. 35.49 – 59.04), in the intervention group, (Wilcoxon rank sum $z = -1.268$, $p = 0.2047$). Age was a significant risk factor: malaria risk decreased with increase in age although SES did not influence the risk of malaria in the model.

Discussion

This randomized controlled trial demonstrated that 15% DEET topical repellents have no effect on malaria incidence transmitted in the early evening. Although there was a consistent decrease in malaria risk among repellent users in both the cluster and household malaria rates, as seen from the results above, this reduction was not significant. This finding is consistent with a study carried out in southern Lao PDR using an identical 15% DEET repellent [35]. It should be noted that, findings from other studies using a higher concentration of 20% DEET with Permethrin in soap that gave over 12 hours of complete protection from mosquito bites [11] and Para-menthane 3–8 diol repellents with close to 100% efficacy for over six hours [30,36] did demonstrate a significant protective effect in Pakistan [11], Bolivia [10] and Ghana [23] and this could be one of the potential explanations for the observation of a treatment effect in these studies. It can be argued that in the Lao-PDR study, 15% DEET provided ~ 100% protection against mosquito bites. However, the number of major malaria vectors, *Anopheles minimus* and *Anopheles maculatus*, caught in entomological collections in the Lao-PDR study was very low and that the effect observed, was probably that of 15% DEET against *Stegomyia* and *Culex* mosquitoes which made up the bulk of the collections. Therefore, as Anophelines are known to show less response to repellents compared to *Stegomyia* and *Culex* mosquitoes [37,38], the repellent effect observed in the Lao-PDR study was greater than at higher densities with a greater proportion of Anophelines as tested in Tanzania [19].

Table 3 Estimated incidence rates by treatment arm and estimated intervention effects

	Intervention arm	Control arm	% Reduction in rates	Wilcoxon rank-sum on residuals (p-value)
Malaria cases	115	137		
ATP analysis				
Individuals randomized	2208	2168		
Households randomized	463	462		
Total person-years	1952.81	1972.38		
Average Household rates/1000 person-years	60.12 (95% C.I. 45.08-75.15)	84.54 (95% C.I. 61.04 108.05)	24.42%	-1.267 (0.2051)
S.D.	164.42	257.07		
Average cluster rates/1000 person-years	60.45 (95% C.I. 48.30 72.60)	68.29 (95% C.I. 37.05-99.53)	8%	0.529 (0.596)
S.D.	16.98	43.66		
ITT analysis				
Individuals randomized	2224	2202		
Households randomized	468	469		
Total person-years	2580.44	2554.92		
Household rates/1000 person-years	47.26 (95% C.I. 35.49-59.04)	68.21 (95% C.I. 49.59-86.84)	20.95%	-1.268 (0.2047)
S.D.	129.60	205.23		
Cluster rates/1000 person months	45.43 (95% C.I. 36.02-59.79)	53.21 (95% C.I. 30.98-104.16)	7.78%	0.227 (0.8206)
S.D.	11.32	34.90		

Power

There are several factors that are likely to have masked any treatment effect in this study, the most likely being the lack of power to discriminate a statistically significant difference between study arms. The lack of power in the study was likely caused by four factors:

First, rapid scale-up of LLINs to achieve universal coverage has been actively taking place in Tanzania [16]. This had led to a substantial decline in malaria in the country and by extension the study area [39]. As a result, the incidence of malaria in the village was likely lower than the incidence assumed for calculation of sample size for this study. This likely led to an underestimation of the sample size required to observe a difference between the two treatment groups. Secondly, during the study period, Tanzania experienced a drought that likely further reduced malaria transmission, and as a result, there were too few malaria episodes in the study area to accurately discriminate any reduction in malaria attributable to the repellent [40], highlighting the need for such studies to be carried out for more than one transmission season to avoid such problems. Third, most of the participants recruited in to the study come from a farming community. Therefore, during the planting and harvesting seasons, these participants relocated to their farmhouses [41]. As a result it was difficult to establish compliance during these periods and those participants

were excluded from the study. This lowered the study sample size further and with it the power to detect a treatment effect. Lastly was the likely overestimation of the assumed malaria incidence in the study area that was used for sample size calculations. Malaria incidence in this study was estimated from reported fever rates in children less than 5 years of age in the study area [26]. Therefore, even though scale up of LLINs and the drought experienced during the study might have lowered the malaria incidence in the study area, it is also likely malaria rates used for estimation of sample size might have been overestimated and hence undermined the study power to observe a difference between the treatment groups.

Compliance

Compliance in this study was measured by self-reporting of use every evening by the household head or a household member that was able to engage with the field workers during the monitoring surveys. However self-reporting is an unreliable measure of compliance, as it have been shown to overestimate compliance [42]. As a result, the ATP analysis used to measure malaria incidence is likely to underestimate the actual malaria incidence in the intervention and control arms, as a larger value of person-time will be used than that of individuals that actually complied to the study reducing discriminatory power. However, if the

randomisation between the two treatment groups was done correctly then the overestimation of compliance and its resultant effect of the study outcome, is likely to be similar in both treatment groups, ruling out the likelihood of overestimation of the treatment effect. This underlines the importance of correctly estimating the compliance in studies of personal protection in order to avoid confounding the outcomes of such studies.

Active versus passive case detection

Due to logistical reasons, this study recruited a single government health facility for collection of clinical data by passive case detection. As a result, the study is likely to have lost malaria cases to the other health facility present in the area. Anecdotally, some participants complained that they went to the other health facility because the study facility always told them that they did not have malaria even though they knew they had malaria, so they did not trust the diagnosis. Also some individuals might have opted to use traditional medicine, treat diseases at home or buy drugs directly from the numerous drug stores in the study area if they felt sick. All these are potential malaria cases that the study might have lost, lowering both the sample size and estimates of malaria incidence in the area. It would have been advantageous to collect data from both health facilities or carry out active case detection. Since malaria was still most common in children under five years in the study site as seen elsewhere [43,44], targeted active case detection in under fives may have gathered more reliable and realistic data on the true impact of repellents in this scenario. Performing supplementary testing of blood spots from all participants attending the health facility with polymerase chain reaction (PCR) diagnosis of subclinical malaria parasitaemia may have also yielded more accurate estimation of transmission prevention by repellents [45].

Sources of bias

Bias was introduced into the study by an imbalance in socio-economic status between the two study groups. The control group demonstrated a higher socio economic status than the control arm. This study however, did not demonstrate a statistically significant association between SES and malaria incidence. However, it is well known that improved housing, whose representative covariates had been adjusted for during analysis, is protective against malaria [46]. A plausible explanation for this is that the participants in this study came from a single village or from villages located closely together. As result they were exposed to the same levels of malaria transmission regardless of their socio-economic status. As socio-economic status is positively associated with seeking treatment at a medical facility [47], it is likely that participants with higher SES sought treatment at the health facility in the

study area at a higher rate compared to participants in the lower SES. Therefore as malaria data was only collected from a single health facility, it is likely that more cases of malaria were observed in participants with higher SES relative to participants from lower SES. Another reason is that no association was seen may be because studies using material ownership as a proxy for measuring SES, to evaluate the relationship between SES and malaria incidence have yielded inconsistent results, at the household level [48].

The study participants were blinded up to some point after allocation of treatments, because of the identical packaging labelled with a three-digit code. However, after a while, field workers reported that study participants in the placebo group complained that they wanted to swap treatment. Participants could differentiate the intervention from the placebo, as mosquitoes would still bite them after applying the 'treatment' while those in the treatment group bragged to their neighbours that they got the good lotion that was effective. This is a source of bias and could have caused treatment contamination between clusters. This problem would have been better overcome with clusters that were geographically isolated, for instance randomization on a village scale, so that individuals were less likely to be able to compare their treatment allocation. Some participants may have sold or given their repellent to relatives in other clusters.

Another potential confounder may have been diversion of mosquitoes from the intervention group to the placebo group. However, this was controlled by allowing for a buffer area of 200 metres between clusters. Diversion in repellent studies has usually been recorded over short distances, one metre [30]. However, distances of 15–20 metres are recommended as the limit for short range attraction of host seeking mosquitoes [49,50] and, therefore, distances of 200 metres between clusters were thought to be adequate to prevent diversion. Treatments were also issued at the household level to prevent intra and inter-household diversion within the cluster. It has been later observed in the study area that mosquito diversion between households does occur [29] and could have confounded data if compliance with the intervention was low by diverting mosquitoes from complying to non-complying households or individuals.

The community was highly knowledgeable about malaria transmission, prevention and control. This is likely a result of the malaria intervention programmes that have taken place in the study village for over two decades [14,17]. The community awareness about topical repellents as a mosquito control tool was poor at the study inception. However, after the study, the community was highly aware of repellents and community members were willing to take up this intervention against malaria if available. This finding demonstrates the feasibility of

topical repellents as a potential tool to supplement LLINs to prevent early evening transmission. In a separate study [Sangoro O, Sarah M, Ann HK, Sarah M: **Feasibility of repellent use in a context of increasing outdoor transmission: A Qualitative study in rural Tanzania, submitted to Malaria Journal for publication**], the community members reported bite avoidance as the major reason for using repellents in the early evenings.

A *posteriori* analysis of data for children under six months was carried out to check whether this age group experienced high malaria transmission because of mosquitoes diverted to them as it was recommended that they not use the repellent [29,30]. This might also have affected the incidence of malaria in the treatment groups if there was uneven distribution of this age category between these groups. However, it was observed that there were only three children and a single case of malaria in this age category, and it can be confidently concluded that this age group did not have any influence on the outcomes observed.

Net usage was also analysed to determine whether there was a difference between the two treatment groups, which would have confounded the outcome. It was observed that reported net usage the previous night was 100% in both treatment groups. These results are presented in detail elsewhere [Sangoro O, Sarah M, Ann HK, Sarah M: **Feasibility of repellent use in a context of increasing outdoor transmission: A Qualitative study in rural Tanzania, submitted to Malaria Journal for publication**].

Recommendations

It was observed that estimation of a sample size with sufficient power was a major shortcoming of this study. Therefore, it is advisable to establish baseline disease incidence rates if a similar study is to be implemented in the future to avoid under powering the study. This can be established from health facility records. However these records may not necessarily be accurate and the more appropriate measure may be to conduct a small cross-sectional or longitudinal survey of the community disease prevalence or incidence and then power accordingly. Another important factor when testing personal protection tools is accurate establishment of compliance. Better methods of establishing compliance are needed. This can be done through frequent follow-up and spot checks or use of indirect methods, such as mosquito saliva antigens, that are a proxy of individual exposure to mosquito bites [51]. Also, development of new tools that require reduced compliance such as long lasting spatial repellents [52] would likely offer greater protection because people often forget to comply daily with a topical repellent unless they feel mosquito bites [53]. Finally, in a time when malaria is becoming more scant due to successful control, active case detection using RDT for

clinical diagnosis followed up by PCR for malaria parasites is most likely the most appropriate means of measuring the impact of additional malaria control tools used in combination with LLINs.

Conclusion

Findings of this trial could not demonstrate if 15% DEET topical repellents had any impact on incidence of malaria transmission in the early evening because the study lacked sufficient statistical power and had several important sources of bias. A better-designed study with sufficient power and fewer sources of bias and ideally a higher concentration of repellent is required to fully understand if topical mosquito repellents are a feasible malaria control tool in the early evenings in Eastern Africa, particularly as repellents have reduced malaria elsewhere in sub-Saharan Africa [23,24]. The acceptability of this intervention is an encouraging finding toward exploring supplementary malaria control tools.

Additional file

Additional file 1: Stata output showing Eigen scores of each variable used in calculation of socio economic status of households.

Competing interests

SO is supported in part by a studentship funded by SC Johnson and Sons through LSHTM. SJM receives consultancy payments to test repellents for a variety of private companies. The other authors declare no conflict of interest.

Authors' contributions

SM and JM conceived and designed the study with inputs from SO. SO implemented the study with help from SM. ET and SO designed the analytical plan and carried out the analysis. SO and SM wrote the first draft of the paper and all the authors revised the manuscript and approved the final version. All authors have agreed to the final version. All authors read and approved the final manuscript.

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Author details

¹Ifakara Health Institute, Box 74, Bagamoyo, Tanzania. ²Disease Control Department, London School of Hygiene and Tropical Medicine, Keppel

Street, London WC1E 7HT, UK. ³Department of Biostatistics and Bioinformatics and Duke Global Health Institute, Duke University, Duke Box 2721, Durham, NC 27705, USA. ⁴Population Services International, Dar es Salaam, Tanzania. ⁵Swiss Tropical and Public Health Institute, Department of Health Interventions, Socinstrasse, 57, CH-4002 Basel, Switzerland. ⁶University of Basel, Petersplatz 1, 4003 Basel, Switzerland.

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RESEARCH

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Feasibility of repellent use in a context of increasing outdoor transmission: a qualitative study in rural Tanzania

Onyango Sangoro^{1,2*}, Ann H Kelly^{2,5}, Sarah Mtali¹ and Sarah J Moore^{1,3,4}

Abstract

Background: Extensive employment of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) has substantially reduced malaria morbidity and mortality in sub-Saharan Africa. These tools target indoor resting and biting vectors, and may select for vectors that bite and rest outdoors. Thus, to significantly impact this residual malaria transmission outdoors, tools targeting outdoor transmission are required. Repellents, used for personal protection, offer one solution. However, the effectiveness of this method hinges upon its community acceptability. This study assessed the feasibility of using repellents as a malaria prevention tool in Mbingu village, Ulanga, Southern Tanzania.

Methodology: Change in knowledge, attitude and practice (KAP) in relation to repellent use was assessed before and after the implementation of a cluster randomized clinical trial on topical repellents in rural Tanzania where repellent and placebo lotion were provided free of charge to 940 households for a period of 14 months between July 2009 and August 2010. Compliance, defined as the number of evenings that participants applied the recommended dose of repellent every month during the study period, was assessed using questionnaires, administered monthly during follow up of participants in the clinical trial. Focus group discussions (FGDs) were conducted in the same community three years later to assess the community's KAP in relation to repellents and preference to different repellent formats.

Results: At baseline, only 0.32% (n = 2) households in the intervention arm and no households in the control arm had ever used topical repellents. During follow-up surveys, significantly more households, 100% (n = 457) in intervention arm relative to the control, 84.03% (n = 379), (p = <0.001) perceived the repellent to be effective. Post-study, 99.78% (n = 462) and 99.78% (n = 463), (p = 0.999) in the intervention and control arms respectively, were willing to continue repellent use. Mosquito nuisance motivated repellent use. From the FGDs, it emerged that most respondents preferred bed nets to repellents because of their longevity and cost effectiveness.

Conclusion: High repellent acceptability indicates their feasibility for malaria control in this community. However, to improve the community's uptake of repellents for use complimentary to LLINs for early evening and outdoor protection from mosquito bites, longer lasting and cheap formats are required.

Keywords: Repellent, Malaria, Knowledge, Attitude, Perceptions, Practice

* Correspondence: psangoro@ihi.or.tz

¹Ifakara Health Institute, Box 74, Bagamoyo, Tanzania

²Disease Control Department, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

Full list of author information is available at the end of the article

Background

Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) have had a great impact on malaria morbidity and mortality in the past decade in sub-Saharan Africa [1-3]. While effective, these tools are intra-domiciliary and predominantly target indoor biting and resting vectors [4]. This favours outdoor resting and biting vectors as IRS and LLINs are less effective against those vectors that exhibit exophily and exophagy [5]. Therefore, as malaria moves from sustained control to elimination, new tools that tackle residual outdoor malaria transmission are needed.

Repellents used outdoors and in the early evenings and mornings, where IRS and LLINs cannot be employed, present one strategy that can be used to push towards the goal of eradication. Topical (skin applied) repellents have been used as a form of personal protection for hundreds of years [6], and have been shown to protect against malaria in South America (80% reduction) [7] and Southern Asia (60% reduction) [8], and more recently in Ghana (34% reduction) [9] and Ethiopia (19% reduction) [10]. The major drawback to using topical repellents is compliance. Topical repellent use requires daily use and frequent re-application as their effects are usually short-lived over a few hours and therewith a change in daily routine (personal behaviour). While changing personal behaviour to use new interventions is not impossible as has been demonstrated in bed net campaigns [11], oral hygiene [12] and hand washing strategies [13], it is influenced by a number of other factors including: cost, perceived quality of the intervention, accessibility, information and ease of use. An intervention is likely to be used by the community if its affordable, perceived to be effective, the community is aware and has knowledge of its uses and finally, the intervention is simple to apply, i.e. it does not require considerable deviation from daily routine [14]. Therefore to influence behaviour change towards uptake of interventions: the community must be educated to improve information on the appropriate measures to employ to prevent disease e.g. use of bed nets to prevent mosquito bites and hence malaria infection. Secondly, the interventions must be made physically accessible to the community, such as considering the distance to shops where bed nets are sold or re-treated. Third, the cost of the intervention must be affordable and perceived as reasonable among community members to encourage use. Perception of the effectiveness of the intervention will also influence uptake, with the community more likely to use interventions they perceive as beneficial to them, for instance LLINs prevent mosquito bites. Lastly, is the ease of use of the intervention being implemented, as the community is more likely to use interventions that require the least deviation from daily routine, like use of drugs with simple dose regimens compared to those that have complicated regimens [14].

Therefore, in an effort to determine the feasibility of using repellents as a mosquito control tool, this study assessed the knowledge/awareness, acceptability, perceptions on effectiveness and preference to different kinds of repellents in a rural community in Kilombero valley, Southwest of Tanzania. The community in this setting has experienced extensive malaria research projects and intervention programmes spanning two decades [15-17] and was expected to be highly knowledgeable about malaria prevention and control. Cooking mainly takes place outdoors and in the early evening, a situation that exposes the community to nuisance mosquito bites and potential malaria transmission before they have employed bed nets. Further, like the rest of sub-Saharan Africa, the study area is experiencing rapid rural development, shifting the spaces and protocols of social behavior. Where once it was customary to retire shortly after sundown, now, owing to rural electrification programmes, residents usually gather in the early evening and stay late into the night at local bars and social centres springing up in the study area, thereby increasing perception of mosquito nuisance and malaria transmission potential at these times.

The dominant vector in this area is *Anopheles arabiensis* [18] that has been shown to shift to early evening and outdoor biting when hosts are unavailable late in the night indoors as a result of high bed net use [19,20]. The presence of rice fields in the study area, as the community's main occupation is farming, provides for a large breeding site of mosquitoes [21]. The presence of this large breeding site is likely increase mosquito abundance in the study area, and with it potential malaria transmission and nuisance biting.

Before the start of the clinical trial, the community were sensitised to the potential for repellents as a malaria prevention tool through skits, community meetings and leaflets. Therefore, they are likely to understand the importance of topical repellents in prevention of early evening malaria transmission potentially occurring in the study area before they go to sleep under bed nets, and are therefore more likely to be receptive to this intervention. Secondly, the customary practice of cooking outdoors as well as presence of electricity exposes this community to nuisance biting in the evenings as a result of the extensive rice fields present in the area, a situation likely to encourage use of repellent. Finally, repellents were provided free so the community were likely to use them and form an opinion on their efficacy.

Methods

Study area and population

This study was conducted in Mbingu village, Ulanga district, Tanzania, situated 55kms west of Ifakara town at 8.195°S and 36.259°E. There is malaria transmission all year round, with peak transmission occurring in the

months of May and June after the long rains. The village experiences an annual rainfall of approximately 1,200–1,800 mm and an annual temperature range of between 20°C and 32.6°C. The village borders an extensive field cleared for irrigation, which provides an ideal breeding site for malaria vectors. The houses in the village are clustered in groups of 3–5 households, which mainly belong to one family, but in a few instances the houses may be rented by different families. In July 2009 (at the inception of the clinical trial), the population of the study area was estimated to be 7,609, with each household having approximately 5 members [22]. Most houses are constructed from mud walls and thatched roof, with one-third made from brick walls and corrugated iron roof.

Outline of study

Between July 2009 and August 2010, a placebo-controlled cluster randomized clinical trial was conducted in the study village where 15% DEET (*N,N*-Diethyl-3-methylbenzamide) topical repellent and an identical placebo lotion were randomly issued to 940 households in the study village [23]. The clinical trial participants were also issued with double size LLINs per sleeping space to ensure equity. Treatments were issued to two study arms of 10 clusters with 47 households each. One study arm was issued with topical repellent lotion while the other study arm received a placebo lotion and both arms were followed up for 14 months to assess the malaria incidence between these two groups. Concurrent with the clinical trial, a knowledge, attitude and practice survey (KAP) of the repellents issued during the clinical trial was conducted by administering a questionnaire (Additional file 1: Repellent KAP survey tool) at the baseline of the clinical trial (before/entry survey) to assess community knowledge of repellents; at the beginning of every month when field workers visited the households to replace repellents that had run out (follow-up survey) throughout the study period, to assess the acceptance and compliance of the community to the repellent issued and perceived effectiveness; and at the end of the clinical trial (after/exit survey) to assess willingness to continue use of repellents. A separate Focus Groups study was conducted three years later in June 2013.

Procedure

Baseline survey

At baseline, written informed consent was sought from the household heads that were willing to participate in the clinical trial. The household heads gave consent for all household members who were below 18 years. Household members above 18 years were asked to sign their own written consent forms. As the household was analysed as a unit, a structured questionnaire of KAP in relation to

repellents was administered to the household head. A unique ID was stapled on the door of each household that was recruited into the study.

Follow-up survey

To assess acceptability and use, at the beginning of every month after the baseline survey, field workers visited the households recruited in the study to replace the tubes of repellent issued the previous month. A KAP questionnaire was administered during these visits, where the households were asked if they liked the repellent issued and their perceptions on the effectiveness of the repellent. The fieldworkers also administered a compliance questionnaire, where household members were asked if any household member had skipped a day of repellent use in the past month and reasons for missing that day. However, if during the follow up survey there were no household members present to answer the questionnaire on compliance, and continued to be absent for seven consecutive days after the first visit to assess compliance, that household was considered non-compliant to repellent use for that month. If the households reported that any household member did not use the repellent, that household member was removed from follow up time for the period they did not use the repellent. Thus, if all household members reported using repellent each night in the past week and an adult member of the household was present to be issued with new repellent, that household was considered compliant for the previous month. In addition, the number of treatment tubes (repellent and placebo tubes) issued per month was recorded, to determine if there was a difference in the number of tubes issued in each month per treatment group. Differences between recalled and observed compliance were not measured.

Post-study survey

At the end of the clinical trial, (August 2010), an exit KAP (post-study) questionnaire to assess perceptions on effectiveness and willingness to pay if repellent was provided at cost was administered. In particular, the respondents were asked what was their perceived cost for the repellent issued during the clinical trial. They were also asked how much they were willing to pay for the tube of repellent they were given during the clinical trial.

Focus group discussions

In-depth discussions

Seeking an in-depth understanding of the knowledge, attitude, perceptions and practice in relation to repellents as a vector control intervention, a descriptive exploratory study, consisting of seven Focus-Group-Discussions (FGDs) and one Small Group Interview (SGI) was conducted in the study village from 10th – 28th June 2013, three years after the clinical trial. The participants may

or may not have participated in the initial clinical study of topical repellents, as prior participation in the previous trial was not an inclusion or exclusion criterion. Several different formats of repellents were provided to participants to measure perceived preferences in delivery formats of repellents among members of a community that had previous familiarity with repellents.

Sampling of FGD participants

This study initially used convenient sampling to enrol household heads in the village. A purposive sample of households with the following characteristic were drawn from the community:

- Households that had the males as household heads.
- Households that had females as household heads (widows, divorced, separated etc.).
- Households that had males as household heads but from which their female partners were invited for the FGDs and SGI.
- Households that had children of school going age (both primary and secondary schools).

From this sample, 6 – 12 individuals from households with each of the above characteristics were interviewed in seven FGDs and one 5-member SGI. The FGDs were dynamic in nature consisting of individuals from 10 to 60 years of age and sampling was stopped at the 'point of saturation' (no further 'new' information generated).

Study tools

Based on literature on knowledge and practice in relation to repellent use and on *a priori* experience of repellent work with the community in the study area, an interview guide on perceptions and practices around repellent use in Mbingu village was developed for conducting the FGDs. This guide was pre-tested on four villagers, two men and two women before undergoing further changes based on the feedback from these villagers. The outcome was a simple interview guide that

consisted of six open ended questions that were structured in a flexible manner to allow for any emerging ideas from the participants to be incorporated there in.

Repellents explored

The different types of repellents issued to the participants of this study were; Permethrin impregnated 'kangas' (a sheet of fabric worn around the waist by women in Africa), 15% DEET (*N, N*-Diethyl-3-methylbenzamide) topical repellent in petroleum jelly format, 15% DEET topical repellent in spray format, 30% PMD (Para-Methane 3-8-diol) topical repellent in lotion format, 30% PMD topical repellent in spray format, 2% transfluthrin impregnated sisal strip (sack), that was hung in a common area where all household members sat, (Figure 1) [24] and 2% permethrin impregnated net fencing that was designed to protect individuals sitting outdoors, especially around the cooking area (Figure 2).

Procedures

Participants were verbally informed on the objectives and aims of the study, its voluntary nature, risks and benefits. Thereafter verbal informed consent was sought from the purposive and final sample of participants after all ethical considerations of the study had been outlined. Interview schedules, including convenient interview times and venues were then negotiated between the study investigators and participants and the study commenced from the 10th to 28th of June 2013. The interviews were all conducted in Swahili and lasted between 30mins and 1 hour in the various local settings preferred by the participants. Consent was sought to use a tape recording device for the sessions with all villagers agreeing to be tape recorded prior to commencement of the interviews. First, four FGDs with the four different respondent groups: households that had the males as household heads, households that had females as household heads (widows, divorced, separated etc.), households that had males as households heads but from which their female partners were invited for interviews, and households that had children of school going age (both



Figure 1 Testing the efficacy of transfluthrin impregnated sisal strip in the semi-field system at the IHI.



Figure 2 Installation of permethrin impregnated fencing around an outdoor kitchen/cooking area in the study area.

primary and secondary schools), were conducted where community knowledge (familiarity) and use of repellents as a mosquito control tool was assessed. At the end of these first four FGDs, the respondent groups were issued with different formats of repellents to use for a week. After using the different repellent formats for one week, these respondents groups were recalled for a further three FGDs and a single SGI where experiences of repellent use and preference to different repellent formats were assessed.

Data management

Data from the baseline, follow-up, and post-study surveys were linked using the household unique identifier. Data from these questionnaires were entered into and coded using an Epi-info template that corresponded to the format of the questionnaires. All data was double entered into Epi-info, where it was checked for excesses or missing of data. Data was then exported to Microsoft Access 2010 database where it was checked for duplicates. Data from the FGDs was collected using tape recorders and imported into the computer where they were stored as audio files ready for transcription and analysis.

Data analysis

All data analysis was carried out in STATA 11.2 (Stata-Corp LP, College Station, Texas, USA) software. Data from the baseline, follow-up and post-study surveys were analysed using descriptive statistics and are presented in tables (Tables 1, 2 and 3).

Data from the socio-economic status (SES) was analysed using principal component analysis (PCA). A socio-economic index was generated using PCA and the generated score used to show wealth index of each household. Indicators of (SES) used were; asset ownership, household construction materials and education level of household head. These results are reported in detail elsewhere [23]. Data for KAP collected during the follow-up survey was analysed by determining trend over time, using descriptive statistics. Compliance data collected using the

follow-up survey was also stratified by SES quintiles to determine if there was a difference in repellents use by SES quintile.

Data for KAP collected at baseline and post-study survey was analysed by comparing the before and after studies using descriptive statistics. Likewise, in the post-study survey, willingness to pay was compared across the SES quintiles.

The number of repellent and placebo tubes issued was analysed by linear regression against month, treatment group and an interaction of month and treatment group to determine if there was a significant difference in the number of tubes issued in each month and per treatment group.

Data collected over the study period (follow-up survey) was used to report outcomes on compliance, community liking the repellent and perception of effectiveness of repellents because it was assumed to be less prone to recall bias compared to data collected at the end of the clinical trial (post-study survey).

Audio files from FGDs were transcribed verbatim in Microsoft Office and imported into Nvivo 9 (QSR international Pty Ltd 2006–2010) qualitative analysis software. The data was then coded into themes as they emerged from the response data in the transcripts. This content analysis also allowed for themes emerging from the data to be considered during iterative coding. The final coding tree (structure of categorizing data) consisted of identified themes from the data as well as unanticipated themes from the respondents. The final stage of the analysis involved re-organization of the themes into larger categories of themes communicating the key messages from each of the smaller themes under them (Table 4).

Ethical consideration

Participants were recruited on written informed consent. Ethical approval for the study was obtained from Ifakara Health Institute (IHI) (IHRDC IRB A46), Tanzanian National Institute of Medical Research (NIMR/HQ/R8a/

Table 1 Baseline perceptions on malaria and repellents

	Repellent n (%)	Placebo n (%)	Totals n (%)	P- value
What is malaria				
Disease	285 (93.44%)	270 (95.07%)	555 (94.23%)	0.397
Don't know	20 (6.56%)	14 (4.93%)	34 (5.77%)	
Causes of malaria				
Mosquitoes	302 (99.01%)	280 (98.59%)	582 (98.81%)	0.634
Other	3 (0.99%)	4 (1.41%)	7 (1.19%)	
Knowledge of malaria prevention methods				
Bed nets	286 (94.38%)	271 (95.42%)	557 (94.89%)	0.664
Environmental management	7 (2.31%)	3 (1.05%)	10 (1.70%)	
Going to hospitals	4 (1.32%)	2 (0.70%)	6 (1.02%)	
Using repellents	1 (0.33%)	1 (0.35%)	2 (0.34%)	
Don't know	5 (1.65%)	7 (2.46%)	12 (2.04%)	
Knowledge of mosquito breeding site				
Water puddle	291 (95.40%)	270 (95.40%)	561 (95.41%)	0.998
Other	14 (4.60%)	13 (4.60%)	27 (4.59%)	
Protection methods used				
Bed nets	294 (95.14%)	277 (96.85%)	571 (95.97%)	0.600
Mosquito coils	3 (0.97%)	3 (1.04%)	6 (1.01%)	
Environmental management	7 (2.26%)	5 (1.74%)	12 (2.02%)	
Covering oneself	4 (1.29%)	1 (0.34%)	6 (0.84%)	
Using repellents	1 (0.32%)	-	1 (0.17%)	
Reasons for using protection methods				
Effective	174 (56.31%)	154 (54.03%)	328 (55.22%)	0.008
Readily available	34 (11.00%)	22 (7.71%)	56 (9.34%)	
Cheap	23 (7.44%)	8 (2.80%)	31 (5.22%)	
Easy to use	76 (24.59%)	100 (35.08%)	176 (29.63%)	
Other	2 (0.64%)	1 (0.35%)	3 (0.51%)	
Reasons for not using repellents				
Don't understand use	139 (45.27%)	118 (41.40%)	257 (43.41%)	0.057
Not aware of repellents	38 (12.37%)	28 (9.82%)	66 (11.15%)	
Not available	109 (35.50%)	115 (40.35%)	224 (37.84%)	
Expensive	16 (5.21%)	24 (8.42%)	40 (6.76%)	
Other	5 (1.62%)	-	5 (0.84%)	
Willingness to use repellents				
Yes	309 (99.67%)	286 (100%)	595 (99.83%)	0.336
No	1 (0.32%)	-	1 (0.17%)	

VOL IX/780) and the London School of Hygiene and Tropical Medicine Ethical Review Board (LSHTM ERB 5174). IHI provided study monitoring.

Results

Baseline survey

At baseline, only 0.32% of the households had ever used repellents in the intervention arm, while no households had ever used repellents in the control arm (Table 1). Two

households reported burning mosquito coils, five households repelled mosquitoes with a smoky fire and one household reported using repellent plants (data not shown). Most households (95.7%) used bed nets as these had been delivered through various governmental and non-governmental schemes from 1997 onwards. When asked about malaria a similar proportion of the households in the intervention and control arms reported that malaria is a disease: 93.44% (n = 285) and 95.50% (n = 284),

Table 2 Assessment follow up of households, repellent use and perceptions during the study period

	Repellent n (%)	Placebo n (%)	Total proportions/treatment	P value
Like repellent				
Yes	462 (99.35%)	390 (84.41%)	852 (91.91%)	<0.0001
No	3 (0.65%)	72 (15.59%)	75 (8.09%)	
Compliant				
Yes	379 (81.50%)	361 (78.13%)	740 (79.83%)	0.202
No	86 (18.49%)	101 (21.86%)	187 (20.17%)	
Perceived effectiveness				
Yes	457 (100.00%)	379 (84.03%)	836 (92.07%)	<0.0001
No	0 (0.00%)	72 (15.96%)	72 (7.93%)	

Table 3 Assessment of perceptions on repellent use, effectiveness and cost after the study period

	Repellent n (%)	Placebo n (%)	Total proportions/treatment	P- value
Reasons for non-compliance				
Forgot	35 (70.00%)	89 (60.13%)	124 (62.63%)	0.241
Away in the field	13 (26.00%)	56 (37.83%)	69 (34.85%)	
Don't like repellent	1 (2.00%)	-	1 (0.51%)	
No mosquitoes	1 (2.00%)	2 (1.35%)	3 (1.52%)	
Ran out of repellent	-	-	-	
Other	-	1 (0.67%)	1 (0.51%)	
Perceptions about repellents				
Effective	455 (98.69%)	208 (45.61%)	663 (72.30%)	<0.0001
Easily available	5 (1.08%)	50 (10.96%)	55 (6.00%)	
Nice smell	-	99 (21.71%)	99 (10.80%)	
Smooth on skin	-	98 (21.49%)	98 (10.69%)	
Other	1 (0.21%)	1 (0.21%)	2 (0.22%)	
Willingness to use repellent again				
Yes	462 (99.78%)	463 (99.78%)	925 (99.78%)	0.999
No	1 (0.21%)	1 (0.21%)	2 (0.22%)	
Willingness to pay				
Yes	458 (99.78%)	455 (98.48%)	913 (99.13%)	0.034
No	1 (0.21%)	7 (1.51%)	8 (0.87%)	
Perceived cost of repellent				
< 0.6 USD	99 (21.80%)	111(26.74%)	210 (24.17%)	0.023
0.6 – 1.2 USD	280 (61.67%)	212 (51.08%)	492 (56.62%)	
1.2 – 1.8 USD	61 (13.43%)	75 (18.07%)	136 (15.65%)	
1.8 – 3.05 USD	13 (2.86%)	17 (4.09%)	30 (3.45%)	
> 3.05 USD	1(0.22%)	-	1 (0.12%)	
Amount participants were willing to pay				
< 0.30 USD	388 (83.43%)	402 (87.77%)	790 (86.06%)	0.347
0.30 – 0.60 USD	64 (13.91%)	52 (11.35%)	116 (12.64%)	
0.60 – 1.20 USD	7 (1.52%)	4 (0.87%)	11 (1.20%)	
1.20 – 1.52 USD	1 (0.21%)	-	1 (0.11%)	

Table 4 Major themes generated from the Focus group discussions (FGD's) and Small group interviews (SGI)

Major results theme	
Theme 1	Respondents were aware of the link between malaria and mosquitoes, but their knowledge on malaria aetiology and transmission was shallow. This did not however, effect their compliance with an intervention that was available free of charge.
Theme 2	Although respondents had adequate knowledge of repellents as a mosquito control tool, they preferred to use the bed net over repellents.
Theme 3	Those respondents aware of topical repellents had adequate knowledge on their proper use
Theme 4	Availability (access) and cost of repellents were major barriers to repellent use after the trial ended and repellents were no longer supplied.
Theme 5	The respondents perceived the repellents to be effective against mosquito bites, mostly in the early evenings.
Theme 6	Respondents recommended repellents be made more available and insecticides (permethrin) used to treat clothing be provided to enable self treatment.

respectively. When asked about malaria transmission, most households in the intervention arm 99.01% (n = 302) and control arm 98.59% (n = 280) reported that mosquitoes transmit malaria. Bed nets were the major prevention tool used in the study village, with a similar proportion of reported bed net use in the intervention 95.14% (n = 294) and control arm 96.85% (n = 277). When households that reported bed net use, were further asked why the preferred bed nets to other tools, a significantly larger proportion cited effectiveness relative to other reasons: 56.31% (n = 174) and 54.03% (n = 154) in the intervention and control arm, respectively. Other reasons for use of bed nets as well as other mosquito bite protection methods are reported in Table 1. It should be noted that the bed nets reported by the respondents, were not those issued during the clinical trial, but they were reporting on tools they used before the onset of the clinical trial. However, bed nets were given at the start of the clinical trial to ensure equity between the study arms. An equal proportion of households in both the intervention 95.40% (n = 291) and control 95.40% (n = 270), arms reported that mosquitoes breed in standing water. The major barrier to repellent use in this community was lack of knowledge on how to use repellents, with 45.27% (n = 139) households in the intervention and 41.40% (n = 118), in the control arm reporting that they did not understand how topical repellents were used. Lack of awareness of repellents was also reported as a barrier to repellent use, with 35.50% (n = 109) and 40.35% (n = 115) of the households in the intervention and control arms respectively, indicating that they were not aware of repellents as a mosquito control tool. However, when repellents were made available knowledge was no longer a barrier to compliance. All households were willing to use repellents to prevent mosquito bites: 99.67% (n = 309) of the households in the intervention and 100% (n = 286), (p = 0.336), in the control arm were willing to use repellents, even though this tool was novel in this community after community sensitization, (Figure 3).

Follow-up survey

A follow up survey was conducted to assess household compliance to repellent use. Compliance in this context is defined as having recalled use of the repellent every night in the past month. However, if during the follow up survey there were no household members present to answer the questionnaire on compliance, and continued to be absent for seven consecutive days after the first visit to assess compliance, that household was considered non-compliant to repellent use for that month. If the households reported that any household member did not use the repellent, the household member was removed from follow up time for the period they did not use the repellent. Reported household compliance with repellent use was not significantly different between the study arms: 81.50% (n = 379) in the intervention and 78.13% (n = 361) in the control arm, (p = 0.202) during the study period. Significantly more households liked using the repellent in the intervention arm 99.35% (n = 462) compared to the control arm, 84.41% (n = 390), (p = <0.0001). When asked about effectiveness, significantly more households in the intervention arm, 100% (n = 465) compared to the control arm 84.03% (n = 379), (p = <0.0001), perceived repellents to be effective (Table 2). Also, significantly more households that perceived the repellent to be effective complied with repellent use (72.31%) compared to those households that did not comply (27.68%), (p = <0.0001). This indicates that relief from mosquito bites was a motivating factor in repellent compliance.

When the perceptions of effectiveness of repellents was analysed over the study period, it was observed that there was an increase in the number of households reporting the repellent to be effective over time. This trend was also observed for households that reported to like the repellents. Compliance was observed to increase over the study period, with more households reporting repellent use at the end of the study compared to the start of the study. Because the repellents were given out



Figure 3 Community sensitization meeting on repellents conducted by the social marketing team from IHI.

for free there was no difference in repellent compliance between the most poor and least poor socioeconomic quintiles ($p = 0.369$), data not shown.

There average number of tubes issued per household was 6.73 (95% C.I. 6.51 – 6.95) and 6.92 (95% C.I. 6.68 – 7.16) per household per month in the intervention and control groups, respectively and there was no significant difference between the treatment arms: Odds Ratio 1.68 (95% C.I. 0.32 – 84.25, $P = 0.803$) and this remained constant for the duration of the study period.

Post-study survey

The main reason for non-compliance to interventions was forgetfulness, with 70% ($n = 35$) of the households in the intervention and 60% ($n = 89$), ($p = 0.241$) in the control arm reporting that the major reason they did not comply with the intervention at some point during the study was because they forgot to apply the repellent. Travel also lead to non-compliance with 26% of households in the intervention arm and 37.83% of households in the control arm not complying for a month because they had gone to work in the fields.

When asked why they liked using the repellents, significantly more households in the intervention arm 98.69% ($n = 455$) relative to the control arm 45.56% ($n = 208$) cited effectiveness, ($p < 0.0001$). It is worth noting that all households who mentioned nice smell and smooth feeling on the skin as reasons for using repellents were from the placebo arm of the trial. When asked if anyone in their household suffered from malaria during the trial, significantly more participants from the placebo arm answered yes: 32.9% versus 15.5%, ($p < 0.0001$).

Equal proportions of households were willing to continue using repellents after the clinical trial (Table 3). When asked if they would be willing to pay if the repellent was made available at a fee, 99.78% ($n = 458$) of the households in the intervention and 98.48% ($n = 455$), ($p = 0.999$), in the control arm reported that they were ready to pay a small fee, with majority of the households in the intervention, 84.34% ($n = 388$) and control arms 87.77% ($n = 402$), ($p = 0.347$) willing to pay at most \$ 0.30 for a tube of repellent (Table 3), even though all participants perceived that the value of the repellent was at least double that figure. There was no difference in willingness to pay when SES quintiles were compared ($p = 0.668$).

Focus group discussions

Perceptions around malaria control and transmission

To provide a general picture of the community's knowledge, attitude and practice in relation to malaria and ways to control malaria, participants were questioned about their knowledge of malaria transmission and methods of prevention and control used. Some of the participants had a comprehensive understanding of malaria and control, as observed from the response of one female respondent below: "*Malaria is caused by a female mosquito when it bites you at midnight*" (Meeting group 5, 16th June 2013).

Interestingly however, and especially in a region where there has been consistent malaria control, research and intervention implementation by both non-governmental and governmental organizations for over 20 years [15-17], the community members did not appear to have an in depth knowledge of malaria transmission. In trying to assess the depth of community knowledge on the malaria

transmission process, the respondents were asked how many times a mosquito had to bite a person for it to transmit malaria. Most of the respondents did not seem to know:

"We do not know unless you tell us"- (Meeting group 4, 14th June 2013).

"Many times"- (Meeting group 1, 14th June 2013).

This indicates the community knowledge on malaria transmission is superficial, so that whilst the community are aware that mosquitoes transmit malaria, their knowledge on this transmission process is scant. These gaps in knowledge might suggest a bias during implementation of malaria control programmes, so that, rather than promoting community sensitization and education on the objectives of the intervention, the link between intervention and disease, and the benefits of the intervention to the individual and the community, these programmes likely focus more on coverage of the control tools.

Preference of malaria prevention tools used in the community

All respondents had used some form of personal protection against mosquito bites even for those who weren't quite sure what malaria was. It also emerged that they had been using these tools for a long time and were convinced that the tool each one of them had been using was the most effective. The most commonly reported malaria prevention tool used was the bed net, when respondents were asked which tool they used to protect themselves from mosquitoes and malaria:

"We use nets" – (Meeting group 1, 14th June 2013).

Even though some of the respondents were aware of mosquito repellents and/or had acquired topical and spatial repellents at some point in the past 2 years, during or after the clinical trial, most of them still preferred using the bed net;

"I would prefer the net" – (Meeting group 3; 25th June 2013)

When the respondents were questioned on why they preferred the bed net to other mosquito control tools, two major reasons were given. The first was cost effectiveness:

"Because mosquitoes will not bite you when you are sleeping under a net but for the repellents they last for a short time and when the smell wears off then the mosquitoes bite you" – (Meeting group 2, 26th June 2013).

The second was generally the ease of use:

"MG: FR: 03: Because it is not cumbersome"- (Meeting group 3, 25th June 2013).

Familiarity of topical repellents

At the onset of the FGDs, most respondents' awareness of repellents was thin, with almost half of them largely unaware of topical repellents as a malaria control tool. However, those who had heard of topical repellents had adequate knowledge on the proper technique of using/ applying the repellents as illustrated by the following quotes when respondents were asked how repellents were used;

"You can apply and then it stays for a few hours after that it is no longer effective and the mosquitoes can bite you. After you apply it you have to wash your hands well with soap" – (Meeting group 5, 16th June 2013).

For those who knew about repellents, the primary source of information was outreach from the Ifakara Health Institute (IHI), previously Swiss Tropical Institute Field Laboratory (STIFL), which is the institute under which the clinical trial project was conducted. When asked how they came to know of repellents most respondents mentioned the clinical trial, which distributed the repellents free of charge:

"They were being distributed by people from STIFL (IHI)" – (Meeting group 1, 14th June 2013).

Reported experience of use after topical repellent distribution and use

After repellent distribution, all respondents reported that they had used the repellent intervention issued to them during the second phase of FGDs. The most commonly reported reasons for continued use of the repellents by the respondents were mainly because of their effectiveness against mosquito bites and also because of the appeal in odour and presentation:

"I liked it because it prevented mosquitoes and its smell did not affect us in any way like causing flu or any other effects"- (Meeting group 3, 20th June 2013).

Another reason that emerged from the interviews was that every member of the household could use the repellent as opposed to other interventions issued which only a few household members used:

"I would choose the applying repellent because it can be used by the children, my husband and even visitors"- (Meeting group 3, 20th June 2013).

There was one report of side effects to repellent use, however this was during the clinical trial and not in the FGD study:

"Yes I know my sibling he used to get rashes all over the body so he was told not to apply the repellents anymore"- (Meeting group 5, 16th June 2013).

Preference for different applications of repellents

After exposing the respondents to typical topical repellents containing active ingredients such as DEET and PMD and in various formats such as lotion, jelly, spray, permethrin impregnated clothing, (*kanga*), transfluthrin impregnated sack cloth and permethrin impregnated net fencing, the respondents expressed the following views and preferences;

"I found the smell to be too strong" when asked about DEET in spray format –(Meeting group 2, 25th June 2013).

"I liked the smell" when asked about PMD in lotion and spray formats – (Meeting group 2, 25th June 2013).

"I did not like the smell because it was too strong" when asked about DEET in jelly format – (Meeting group 2, 25th June 2013).

"The applying repellent because everyone can use it but the kangas cannot be used by everyone" when asked to choose between topical repellents and insecticide treated clothing '*kanga*' – (Meeting group 3, 20th June 2013)

"If you sat near the sack repellent then the mosquitoes couldn't bite you but if you sat just a distance away then they would bite"– when asked about the transfluthrin impregnated sack, (Meeting group 6, 20th June 2013).

"I got the net so I used to sit inside it and the mosquitoes were very few. They used to bite the feet only but I could stay for like half an hour without bothering with any mosquitoes" – when asked about the permethrin impregnated net fencing, (Meeting group 6, 20th June 2013).

Factors that determine the continued use of topical repellents

For those who did not use repellents during the clinical trial, repellents were generally not popular. There were a several barriers to repellent use in this community; the first being access to the repellents:

"We were given repellents for applying but after they got finished I have not used anything else apart from nets" – (Meeting group 1, 16th June 2013).

Repellents were provided of free during the clinical trial. However after the clinical trial, the community was unable to access repellents as they were not available in shops and drugs stores in the study area, as was highlighted by the respondent above.

The costs of the repellents according to most respondents limited their affordability with most respondents prioritizing other living essentials over the repellents. When asked to choose between buying a soda or the repellent (subject to availability), most of the respondents opted to buy the refreshment:

"I would buy the refreshment or a net otherwise I would just use a lot of clothing to cover myself" – (Meeting group 1, 16th June 2013).

Community recommendations on improving repellent use

In an effort to understand how to improve the use of repellents, participants were questioned on what they felt was necessary to make the interventions better. While most of the responses revealed that the repellent application was fine the way it was, other recommendations included the cost of the repellent:

"I wouldn't buy them because that is expensive unless you sold them in 500 shillings bottles" – (Meeting group 1, 16th June 2013).

It should be noted that the bottle the respondent was recommending to be sold for 500 TZS/\$0.30 contained 120 ml of repellent.

Odour of DEET repellent:

"I did not like the smell because it was too strong" – (Meeting group 2: Male respondent).

Issuing extra insecticides so that they could re-treat the impregnated clothing issued:

"I also think that you should give us repellents for the kangas so that we can treat them once we wash them" – (Meeting group 3, 25th June 2013).

Discussion

Despite the proven efficacy and acceptability of repellents for prevention of malaria [7-10], knowledge and utilization of repellents as a malaria control tool is low in sub-Saharan Africa. Lack of awareness of repellents as a malaria control tool is one of the major barriers to repellent use in sub-Saharan Africa. As observed from the baseline survey at the start of this study, most respondents had not used repellents before the implementation of the clinical trial. Therefore, use of topical repellents was completely new in this community as similar to several other studies

conducted in the African continent [25-27]. It is evident that improving community knowledge and awareness [26-29] as well as retooling interventions to community needs and preferences [30] will improve the acceptability and uptake of interventions being advocated. The most commonly used malaria control tool in the study area was bed net. Social marketing of LLINs started in Kilombero and Ulanga district in July 1997, under the KINET project. At the launch of this programme the community was educated on malaria transmission and control [15]. This campaign was followed by the launch of the Tanzania National Voucher System (TNVS), implemented by the National Insecticide Treated Nets programme (NATNET), under the National Malaria Control Programme (NMCP) of Tanzania, from 2004. In 2007, the Ministry of Health and Social Welfare (MoHSW), collaborating with other partners launched the under five Catch-up Campaign, parallel to the TNVS programme. In 2008, the MoHSW and partners launched the Universal Coverage Campaign (UCC) [31]. Therefore, if repellents and indeed any other novel tools are to be accepted and used to complement LLINs and IRS, there will be a need for social marketing, community education and sensitization to be employed for a substantial period of time. It is also essential to determine community preferences. Tools that require daily compliance are initially likely to have limited uptake, as the community has to remember to adhere to them on a daily basis. As observed from the FGDs study, ease of use was one of the reasons why the community preferred bed nets to repellents. This was because, once hung, the bed net was used over a long period of time as you simply pull it down when you get into bed, compared to having to remember to apply the repellent every evening. However, ease of use was not the only factor that effected compliance. In the follow-up surveys, it was observed that there was lower compliance in the control arm relative to the intervention arm. Likewise, in the after study, it was observed that more households in the intervention arm relative to the control arm used the repellent because it was effective. This finding demonstrates that compliance to interventions does not only depend on its availability and ease of use but also on its effectiveness.

In the FGDs it was also observed that even though sisal impregnated sisal strips did not require daily compliance and were easy to use, they were reported to be effective over very short distances, and this discouraged the community from using it.

These finding demonstrates that to impact compliance, the efficacy of the tools being recommended need to be established. A recent mathematical model demonstrated that the effectiveness of any repellent is extremely dependent on two factors: efficacy and compliance (Moore and Briet, in preparation). The most effective tools are

those that have high efficacy and require little user compliance such as house screening [32].

The major reason for use of topical repellents by the community in Mbingu village is to prevent nuisance biting by mosquitoes. Although a proportion of the community could associate mosquito bites with malaria, the results of this study imply that they used repellents to avoid being bitten by mosquitoes rather than to avoid contracting malaria. These results were similar to a study carried out in a coastal community in Mexico, where 80% of the respondents said they allowed IRS in their households to reduce mosquito bites while only 2% said they allowed IRS to avoid contracting malaria [33]; and in rural Tanzania, where respondents reported that main reason for using LLINs was to prevent mosquito bites: 73% of the respondents reported they allowed IRS in their households to reduce mosquito bites and only 17% related protection from mosquito bites with reduction of malaria in the family [25]. These findings demonstrate that tools being advocated as interventions, especially in malaria control should address both short and long-term goals, i.e. address the problem of nuisance biting or mosquito densities (efficacious to enhance uptake) as well as reduce disease prevalence/incidence in the long run (resultant effectiveness). This is likely to encourage uptake and acceptability as opposed to tools whose benefits are realized in the long-term, and highlights the need to test new vector control interventions against nuisance biting insects as well as target vectors during development for a better understanding of how effective that tool will be in the real world for disease control purposes.

The major reason for not using repellents in this community was reported to be lack of knowledge of repellent use and is similar to findings in other studies [26], where low repellents use was associated with poor knowledge of repellents. Availability of repellents in this community was another barrier to repellents use as observed from the baseline survey.

Also, in the FGDs, after the clinical trial, when asked why they did not use repellents, the respondents cited availability as a barrier, reporting that they did not know where to access repellents. Observations carried out by the study investigators during the clinical trial and FGDs, indicated that no topical repellents were available in the shops and drug stores the study area. Therefore, despite most households indicating willingness to continue repellent use, and even pay a small fee, access to repellent was a major barrier to repellent use.

Another barrier to the use repellents was cost [34]. During the FGDs, even though all respondents were aware of repellents as a mosquito control tool, they all preferred using LLINs as they reported that repellents were more expensive in the long run because they had

to be replaced every end of the month compared to LLINs, which could last up to five years before replacement, if well taken care of. This finding was consistent with outcomes from other KAP studies assessing uptake of interventions [25,28,35]. As seen from the above studies, cost of mosquito control interventions greatly influences the acceptability and uptake in communities where they are to be employed. In rural and urban areas in Tanzania, a 150 ml bottle of 15% DEET repellent costs USD \$1.00. On average, respondents were willing to pay \$0.32 for a 150 ml tube of repellent that would last one adult less than one month. The current price of repellents is too expensive for the subsistence farmer, who lives on \$1.50 USD per day. Therefore, even though incorporation of repellents into malaria control programmes on a community scale, is likely to use a cheaper but efficacious option of repellent, as was the case in Ghana [10], it is unlikely that the repellents would be subsidized down to or lower than \$0.32. Also, scale up and extensive use of repellents under programmatic conditions as well as emergence of a repellent market is bound to drive the cost of repellents down. However these cost are unlikely to be lower than the cost of delivering a single LLIN, which costs USD \$5.30 and protects two people for up to 5 years (\$0.50 per person per year) [31]. Therefore if we are to encourage up take of repellents as a malaria control tool, the cost needs to be greatly reduced, potentially through government and non-governmental organizations offering subsidies on repellents following the example of LLINs [23]. The government may also encourage local production of repellents through tax exemption for local repellent manufacturers.

From the FGDs, it was observed that knowledge on malaria transmission and control was relatively superficial. While most respondents associated mosquitoes with malaria, when probed, few were able to detail processes of transmission, aetiology and prevention in any depth. Therefore, although all respondents from the FGDs reported that they used the repellents issued, it is likely that they did so with only a superficial understanding of the objectives of using repellents. This might have been because the community were more concerned with preventing mosquito bites than contracting malaria, as observed in other studies [25,33]. As all respondents reported that they had complied with repellent issued it was not possible to assess the relationship between compliance and level of knowledge of malaria transmission. The superficial knowledge of malaria transmission observed in this community underscores the importance of incorporating community education and sensitization before implementation of any intervention to achieve its desired objective. Social marketing the product, and neglecting key messages regarding how these interventions benefit the communities in which they are being implemented, is likely to negatively effect uptake of that

intervention. It is therefore essential for the community to be involved in designing and implementation of intervention programmes so that they have a better understanding of the objectives and use of tools being employed.

Several studies have shown that there has been better uptake of interventions in communities where awareness and sensitization have been conducted [36]. Promoting knowledge and awareness also deters any misconceptions that the community may have towards a particular intervention and it is essential for effective implementation of that intervention [37,38]. During FGDs for this study, some respondents reported that they had 'heard' that LLINs caused infertility and also claimed that if/when they use repellents then their skin pores will be blocked and they will get sick. However in a KAP study in Rukungiri, Uganda, women who had previous knowledge of the use of ivermectin were more involved in making decisions of how ivermectin should be distributed to the community compared to those women who had no prior knowledge of this drug [39]. It is therefore essential to acknowledge and address the community's misconceptions and misinformation about intended interventions in order to improve acceptability, uptake and effectiveness. Rather than the implementing organizations solely marketing the product to achieve extensive coverage, it is beneficial to also educate the community on the safe use of these interventions and the correlations between their products, the disease and its benefits.

The respondents' preference of LLINs to repellents is attributable to cost effectiveness, convenience of use and availability. The major reason given for non-compliance to repellent use was that the respondents 'forgot' to use it, while ease of use was ranked second among reasons why respondents preferred using bed nets. It was cumbersome to remember to re-apply the repellent after every few hours, unlike simply sleeping under a LLIN. Repellents should therefore be presented in a format that will encourage uptake. As the major occupation in the study area is subsistence farming, most community members bathe in the evening after coming from their farms. Repellents can be incorporated into body lotions so that they are applied after taking the evening bathe. Repellents can also be impregnated in clothing, especially in *kangas* used by women in the evening when cooking outdoors. Development of tools that do not require daily compliance such as long lasting spatial repellents that act over long distance should also be explored [40].

Respondents also preferred LLINs because it protected them when they were asleep and vulnerable to mosquito bites as opposed to when they were awake and could chase mosquitoes away. The community however preferred to use repellents in the early evenings when sitting outside their houses to have a chat with other family members and friends without being bothered by mosquito

bites. This finding is important because it suggests that repellents can be used complimentary to LLINs in the early evening, before LLINs are employed, which was a major objective of this study.

Perceived irritating odour of DEET topical repellents reduced its use by the community in this study, a finding similar to studies in North Tanzania and Mexico where participants refused to use IRS because of the 'bad smell' of insecticides used [25,33], emphasizing that interventions should be tailored to be perceived as pleasing by users. PMD was perceived as pleasant as found in several other studies [7,10,41].

The most salient recommendation that came out of this study was that interventions advocated to the community should fit the community needs, such as providing repellents that have a pleasant smell and feels good when applied to the skin. Respondents that were issued with Permethrin impregnated *kangas*, reported that even though effective, it only protected a single individual at a time and suggested that all members in the household be issued with a treated *kangas*, and like LLINs, be issued with the 'chemicals' (insecticides) used for re-treatments so that when the effect of the insecticide was diminished they could treat the clothing on their own. Insecticide Treated Clothing (ITC), has been successfully implemented in other settings [42-45] and therefore this tool would easily be introduced in this community. Another outcome of this study was the effectiveness of the topical repellents that were issued. The respondents found topical repellents to be effective in protecting against early evening biting outdoors. This finding is similar to other studies, where repellents have been used to protect against vectors biting outdoors and by extension reduce the incidence of malaria [7,8,46]. Therefore both topical repellents and ITC, if designed to meet the needs and preferences of the community, could offer potential interventions that could be introduced for malaria control and would be readily accepted by the community.

Conclusion

In this setting, the major limitations to use of repellents, similar to those identified from other studies were lack of knowledge, availability of repellent, cost and need to remember to use it every evening or even more than once in a single evening. While the community was highly knowledgeable about malaria, their knowledge was found to be superficial, indicating poor community education and sensitization. Although currently LLINs are the most commonly used and preferred malaria prevention and control tool, their introduction to the community was initially marked by similar limitations emerging from this study such as the need to use it daily and the cost being prohibitive. When repellents were provided free of charge to all trial participants compliance was high. It is therefore

likely that uptake will improve if accessibility of repellents is improved through lower costs and greater availability through the commercial sector; comprehensive social marketing and community sensitization on use of repellents, as well as delivery of repellents in formats that respond to community desires. Even though LLINs were the preferred mosquito protection tool, the community saw a benefit in the use of topical repellents in the early evening, especially to prevent mosquito nuisance indicating the potential of using repellents complimentary to LLINs. However, longer lasting repellents are an essential requirement to avoid the need for frequent reapplication that most people find off-putting. The difference in compliance reported during and after the study is likely due to recall bias at the end of the study. Other avenues such as long lasting spatial repellents might be used if they are effective enough to protect the peridomestic space occupied by the family and visitors in the evening.

Limitations to the study

A ranking of repellent preference had previously been reported in this study, but as there were too few repellents types/formats to issue to each FGD participant, these results were discarded along with some themes that had earlier been reported as they did not represent true results of community preference to different repellent formats.

As the participants were only issued with one repellent, it was not possible to explore whether the participants would use the repellents complimentary to each other if they had been issued with different formats of repellents. However, findings from the FGDs indicated the community members used the tools complementarily.

Another limitation of this study is that compliance, during the follow up and post-study surveys, was established by self-reporting of use by the study participants. It was not logistically possible to observe compliance of households to repellents use for each household every evening and therefore observed and reported compliance could not be compared, and this should be taken into consideration when interpreting the results of this study.

Additional file

Additional file 1: Repellent KAP survey tool.

Competing interests

SO is supported in part by a studentship funded by SC Johnson and Sons through LSHTM. SJM receives consultancy payments to test repellents for a variety of private companies. The other authors declare no conflict of interest.

Authors' contributions

SO and SJM conceived and designed the study. SO implemented the study with help from SJM. SO designed the analytical plan and carried out the analysis. SO wrote the manuscript with inputs from AHK and SJM and all the authors revised the manuscript and approved the final version.

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Author details

¹Ifakara Health Institute, Box 74, Bagamoyo, Tanzania. ²Disease Control Department, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK. ³Swiss Tropical and Public Health Institute, Department of Health Interventions, Socinstrasse, 57, CH-4002 Basel, Switzerland. ⁴University of Basel, Petersplatz 1, 4003 Basel, Switzerland. ⁵Department of Sociology, Philosophy and Anthropology, University of Exeter, Byrnie House, Exeter EX4 4PJ, UK.

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Evaluation of Repellent Efficacy in Reducing Disease Incidence

Sangoro P. Onyango and Sarah J. Moore

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INTRODUCTION

Repellents are currently used by millions of people worldwide to prevent nuisance bites from blood-feeding insects, and it is now a multi-million-dollar global industry.¹ Until recently, there was limited scientific evidence on the efficacy of repellents to reduce disease. However, several groups of animals, including passerine birds and white-faced capuchin monkeys, anoint themselves with leaves, fruit, and even millipedes that contain compounds that are proven deterrents of ticks and

mosquitoes.²⁻³ This behavior is observed to increase at times when attacks from such arthropods are higher, as observed in capuchin monkeys of South and Central America.⁴ This fascinating observation is an indication that the use of personal protection from blood-feeding arthropods must improve the biological fitness of the animal that applies such repellents by reducing energy expended on “host defensiveness” or reducing its susceptibility to arthropod-borne diseases.⁵

Although the inhabitants of tropical countries with low per capita incomes may still use smoke and plant materials to keep biting arthropods at bay, the majority of research into the highly effective mosquito repellents that are available today has been carried out by scientists employed by or funded by the military to protect troops stationed in high-disease-risk areas. Some of the world’s most important programs involved in the understanding and prevention of arthropod-borne diseases have risen as a result of conflicts in tropical regions that lead to massive loss of life from diseases such as yellow fever, louse-borne typhus, and malaria.⁶ Two of these discoveries, *N,N*-diethyl-3-methylbenzamide (deet), which is a topical repellent,⁷ and long-lasting permethrin-treated clothing,⁸ are reviewed in this chapter. Two other repellents are also reviewed: *p*-menthane-3,8-diol (PMD), a topical repellent discovered in China,⁹ and mosquito coils that were developed by the private sector in Japan¹⁰ are examples of area or spatial repellents (see the section “Mosquito Coils”).

Topical repellents are oils or lotions applied to the exposed skin or clothes of the consumer, with the most safe and effective being deet, picaridin, and PMD. Picaridin will not be reviewed here, because there is, to date, no epidemiological evidence of its efficacy, although a well-designed trial to evaluate its efficacy against malaria is currently underway with results available in 2014.¹¹ Permethrin-treated clothing is impregnated with a safe pyrethroid insecticide and binding agent to allow the permethrin to adhere to the fabric even after several washes. Permethrin is a synthetic pyrethroid, which has been extensively tested by the military,¹²⁻¹⁵ and is the only insecticide approved for this use category by the U.S. Environmental Protection Agency.¹⁶ It is nonstaining, odorless, and resistant to ultraviolet light and safe for regular use as an excellent tool for long-term prevention of arthropod bites. Mosquito coils are spirally shaped coils made from organic fillers, binders, and additives that allow the organic components to smolder evenly and continuously, to which a volatile pyrethroid insecticide is added that evaporates as the coil smolders over several hours after it is ignited. They are classified as area (spatial) repellents. Spatial repellency is used here as a general term to refer to a range of insect behaviors induced by airborne chemicals that result in a reduction in human–vector contact. This can include knockdown, interference with host detection (attraction–inhibition), or movement away from a chemical stimulus.¹⁷ Other forms of spatial repellents include vaporizers and mats that have available extensive phase II (laboratory) data demonstrating excellent efficacy¹⁸ but no epidemiological evidence of efficacy to date.¹⁹ Vaporizers and mats require electricity to evaporate the insecticide from a small liquid reservoir containing the insecticide and a cellulose mat impregnated with the insecticide, respectively. This feature limits their application for disease prevention in the rural tropics where the majority of vector-borne diseases occur, because electricity is not available. Another intervention of note is passive emanators that have a large surface area, allowing the passive diffusion of insecticides from the surface. There is extensive evidence from studies with dichlorvos that passive emanation of insecticides is effective against malaria vectors (Table 7.1). However, dichlorvos does not have a suitable toxicity profile for public health use.²⁰ The discovery of the extremely nontoxic pyrethroid insecticides metofluthrin and transfluthrin (reviewed in the section “Mosquito Coils”) means that passive emanation of such compounds is an area of current research interest^{21,22} and large-scale epidemiological trials regarding this topic will begin in the near future. This has been publicized on the Notre Dame website (<http://news.nd.edu/news/46769-second-largest-research-award-at-notre-dame-fights-malaria-and-dengue-fever/>). Development of such products will be of great value because although the pyrethroid insecticides used in coils are not harmful to humans, often the smoke produced from the combustion of coils is a nuisance to people, reducing consumer acceptance, and some brands generate products of incomplete combustion, which are harmful to humans.^{23,24}

Table 7.1 Overview of Insect Vectors of Disease, Their Behavior, and Means of Preventing Bites

Vector	Disease	Location	Time of Biting	Indoors/ Outdoors	Transmission Season	Recommendation
<i>Anopheles</i> mosquitoes	Malaria	SSA	Dusk– to dawn with late night peak	Indoors and outdoors	All year with peak during and following the rainy season	Avoid mosquito bites especially after sunset by using insect repellents containing deet or PMD and long clothing impregnated with permethrin. Sleep beneath insecticide-impregnated bed nets. Sleep in air-conditioned/screened rooms where possible, and use mosquito coils containing transfluthrin, D-allethrin, or metofluthrin, if possible outdoors after dark
		SA	Dusk to dawn with early evening peak	Mainly outdoors	During and following the rainy season	
		CA		Indoors and outdoors		
		SEA				
		SCA				
<i>Aedes</i> mosquitoes	Dengue fever	Mainly SSA (75% of cases) some SCA, CA, SA—mainly in residents and long-term travelers >1 month	Dusk to dawn with late night peak	Indoors and outdoors		
		SSA	Daytime and early evening	Indoors and outdoors	All year round, but especially following the rainy season and during epidemics	Prevention of mosquito bites during daytime using a repellent with deet or PMD is essential during epidemics. Use of mosquito coils or heated mats indoors and sleeping in screened accommodation is advised
		SCA SEA CA CAR SA				

(continued)

Table 7.1 Overview of Insect Vectors of Disease, Their Behavior, and Means of Preventing Bites (continued)

Vector	Disease	Location	Time of Biting	Indoors/ Outdoors	Transmission Season	Recommendation
	Rift Valley fever	SSA, ME			During epidemics related to very high rainfall. chikungunya entering EU with climate change	
	Chikungunya	SSA, NAF & ME, SEA, EU				
	Yellow fever	SSA, SA			Can occur year round, but mainly during and following the rainy season and during epidemics	Ensure vaccination for yellow fever before traveling to endemic areas
<i>Culex</i> mosquitoes	Japanese encephalitis (JE)	SEA		Mainly outdoors	All year round, risk mainly among residents and travelers to rural areas	Ensure vaccination for JE before traveling to endemic areas. Avoid mosquito bites especially after sunset by using insect repellents containing DEET or PMD and long clothing impregnated with permethrin. Sleep beneath insecticide-impregnated bed nets. Sleep in screened rooms where possible and use mosquito coils containing transfluthrin, d-allethrin, or metofluthrin if possible outdoors after dark
	Lymphatic filariasis	SSA, SCA, SEA, SA (Haiti, the Dominican Republic, Guyana, and Brazil)	Dusk to dawn with early evening peak	Indoors and outdoors	All year round, risk mainly among residents and long-term travelers	
	West Nile fever	SSA, NAF & ME, SCA, NA, EU			All year round in tropics, warmer months in northern hemisphere	

Sandflies	Leishmaniasis	SSA, SCA, CA, SA 90% of visceral leishmaniasis cases occur in India, Bangladesh, Nepal, Sudan, Ethiopia, and Brazil; 90% of cutaneous leishmaniasis cases occur in Afghanistan, Algeria, Iran, Saudi Arabia, Syria, Brazil, Bolivia, Colombia, and Peru	Most species are active at dawn and dusk and during the night, but in forests and dark rooms they may also attack in the daytime	Most species feed outdoors, but a few feed indoors	All year round	Use long clothing in areas where sandflies are common, as their short mouthparts cannot bite through clothes. Avoid sandfly bites, particularly after sunset, by using insect repellents containing DEET or PMD and by wearing long clothing impregnated with permethrin. Sleep under insecticide-impregnated bed nets (small mesh) and in screened accommodation if possible
Blackflies	River blindness	Mainly SSA (West Africa) also CA, SA, mountainous wet areas, and southern Yemen	Daytime	Outdoors	All times of the year, but more common in residents and long-term travelers >3months	Avoid areas where blackflies are active—near large and fast-flowing rivers. Wear long, light-colored clothing treated with permethrin if habitat cannot be avoided
Deer flies	Loiasis	SSA (West and Central Africa) in rain-forested areas	Daytime	Outdoors	All times of the year but especially the rainy season and more common in residents and long-term travelers	Avoid areas where deer flies are active—near muddy rivers. Deer flies are attracted to wood smoke, so avoid campfires. Wear long clothing. Treat clothing with permethrin if habitat cannot be avoided
Biting midges	No disease, but severe nuisance	AU, NA, CA, EU Most northerly temperate regions	Crepuscular during dawn and dusk, but for most species biting activity peaks in the early evening. Biting in the daytime if conditions are humid, still, and cloudy	Outdoors	Spring, summer, and autumn when adults are present	Avoid areas where midges are active—breeding grounds are acid soils, boggy soils, or coastal salt marsh. Use repellents of choice. Wear midge hoods. Wear long, light-colored clothing and treat clothing with permethrin if habitat cannot be avoided

(continued)

Table 7.1 Overview of Insect Vectors of Disease, Their Behavior, and Means of Preventing Bites (*continued*)

Vector	Disease	Location	Time of Biting	Indoors/ Outdoors	Transmission Season	Recommendation
Tsetse flies	African sleeping sickness	SSA mainly Tanzania, Uganda, Malawi, and Zambia (East African form) Democratic Republic of Congo, Angola, Sudan, Central African Republic, Chad, and northern Uganda (West African form)	Daytime. East African tsetse prefer wooded thickets and west African tsetse are found in forests and vegetation along streams	Outdoors	All times of year	Avoid wearing dark blue or black clothing. Keep car windows closed when traveling through areas of woodland. Wear long permethrin-treated clothing if outdoors in tsetse habitats
Triatomine bugs	Chagas disease	CA and SA, mainly Bolivia	Night	Indoors in rural forested areas, especially in poor housing (mud walls and thatched roofs)	All times of year	Sleep under insecticide-impregnated bed nets. Move the bed away from the wall
Fleas	Plague	SSA, SCA, NA	Day or night	Indoors or outdoors	All times of year	Avoid areas of high rodent density (primary host). Wear a repellent containing deet and tuck trousers into socks to avoid bites around the ankles. Use an insecticide-treated bed net if sleeping in endemic areas
Hard ticks	Tick-borne encephalitis (TBE)	SCA, EU, SEA (China and Korea)	Day and night	Outdoors	Tropics: any time Temperate: spring and summer, although season extending due to climate change	Vaccine available in Europe and Canada, but not licensed for use in the United States Avoid areas where ticks are abundant in woody and bushy areas with high grass and leaf litter. Walk in the center of trails Examine clothes and skin for ticks regularly (at least daily), and remove them with forceps Wear long clothing and tuck clothing into boots Use repellents containing deet and permethrin on clothing

Rickettsial diseases including spotted fevers and Q fever	SSA, SCA, SEA, NA, EU				
Tularaemia	SSA, NA, EU				
Lyme borreliosis	SSA, SCA, SEA, NA, EU				
Soft ticks	SEA	Any time	Outdoors	All times of year	As for ticks
Chigger mites					

Note: SSA, Sub-Saharan Africa; NAf & ME, North Africa and Middle East; SCA, South Central Asia; SEA, Southeast Asia; AU, Australia; PI, Pacific Islands; NA, North America; CA, Central America; CAR, Caribbean; SA, South America; EU, Europe.

The annual market value of personal protection consumer products is over \$2 billion for powders, gels, and repellents and \$2.6 billion for spatial repellents including vaporizers and coils. It is estimated that 45–50 billion mosquito coils are used annually by approximately 2 billion people worldwide,²⁵ mainly in Southeast Asia, but with a growing market in South America and Africa. These products present a great opportunity for public health, because such products could provide a means of disease control that is already proved to be highly acceptable to end users, because those who can afford them are willing to buy them.

VECTOR BEHAVIOR MODIFICATION FOR DISEASE PREVENTION

The World Health Organization (WHO) has recommended that all travelers to disease-endemic areas should minimize exposure to insect bites by selecting a combination of personal protection methods including insect repellents, mosquito nets, mosquito coils, aerosol sprays, protective clothing, screening, and air-conditioning.²⁶ The U.S. Department of Defense spent \$4 million in developing the insect repellent system that comprises the proper wearing of a permethrin-treated uniform, and the application of extended-duration deet lotion to exposed skin that, if used correctly, provides close to complete protection from arthropod-borne diseases.²⁷ However, there has been no discussion on the implementation of repellents for public health use. The main explanation behind this is that until recently there were insufficient studies conducted to convincingly demonstrate that repellents can be effective against disease transmission.

Public health vector control tools such as indoor residual spraying (IRS) and the use of long-lasting insecticide-treated nets (LLINs) are extremely effective in sub-Saharan Africa.²⁸ Massive mobilization of both financial and political resources of the past decade²⁹ has resulted in the scale-up of LLINs and IRS and has had a great impact on malaria transmission.²⁸ However, there is a substantial amount of disease transmission both within and outside of Africa,³⁰ where vector behavior evades control through conventional means such as insecticide-treated materials because vectors bite outdoors and at times when people are still active (Tables 7.2 and 7.3). Recent estimates are that 16% of global malaria burden and 8% of malaria mortality occur outside of Africa, whereas outbreaks of dengue and other arboviruses are increasing and spreading geographically.³¹ Thus, tools targeting these outdoor and day biters are required. With the new impetus for malaria eradication of the past decade and the realization that the existing control tools LLINs and IRS cannot solely achieve this, repellents are increasingly being considered as the supplementary tool in appropriate scenarios.³² Modern repellents are extremely effective in preventing human–vector contact. The burden of vector-borne disease remains elevated despite substantial gains in control. There remains a challenge to develop repellency as a vector control option to complement existing tools in scenarios where the vector³³ (Table 7.1) or the human population³² (Table 7.2) exhibits behaviors that require their use.

How Repellents Work to Reduce Vectorial Capacity and Vector-Borne Disease

When considering vector control for disease prevention, it is useful to consider how repellents could reduce the vectorial capacity (VC) of the disease vector population of interest and thus reduce disease transmission. The concept of VC was derived from models of malaria transmission first devised by Ross and was developed to guide the first global malaria eradication plan.³⁴ VC is described by an equation (Box 7.1) and is defined as “the average number of inoculations with a specified parasite, originating from one case of malaria in unit time, that the population would distribute to man if all the vector females biting the case became infected.”³⁵ The concept of VC is sufficiently simple that it can be applied with some modifications to account for varying vector behavior, competence, and ecology, as well as differences in the dynamics of infection, disease, and immunity in vertebrate hosts, and has been used to

Table 7.2 Human Behavior That Necessitate the Use of Complementary Control Tools

Activity	Region	Disease	Increased Risk of Disease	Vector	Reference	Prevention Strategy	Reference
Illegal gold mining	Amazon (Brazil)	Malaria	Prevalence of malaria among individuals involved in gold-mining activities (67%) OR = 1.92 (1.05–3.50), who came from nonendemic areas (43%) 1.56 (1.06–2.29), and who reported being outside after 5 PM (37%) 2.04 (1.06–3.95)	<i>Anopheles darlingi</i>	43, 44	Health education, provision of free repellents, and/or permethrin-treated clothing plus long-lasting insecticide-treated hammock nets to miners. Topical repellents in this region prevent 80% of malaria among users in the Bolivian Amazon OR = 0.20 (95% CI = 0.11–0.38) Permethrin-treated uniforms prevented malaria OR = 0.24 (95% CI = 0.07–0.87) among soldiers in Colombia—part of the Amazon region	45, 46
Open gold mining	Amazon (Bolivar state, Venezuela)	Malaria	Malaria was almost absent until the beginning of mining activities in the 1980s. Now, between 2001 and 2010, 72.3% (22,746 cases) are among men of working age mainly from mining camps	<i>Anopheles darlingi</i> and <i>Anopheles marajoara</i>	47, 48		
Overnight forest activities, e.g., hunting and travel	Amazon (French Guiana)	Malaria	3.3, 95% CI = 1.1–9.5	<i>Anopheles darlingi</i>	49		

(continued)

Table 7.2 Human Behavior That Necessitate the Use of Complementary Control Tools (continued)

Activity	Region	Disease	Increased Risk of Disease	Vector	Reference	Prevention Strategy	Reference
Agricultural expansion into forested areas	Amazon (Brazil)	Malaria	Possibly as a result of their more frequent involvement in forest-related high-risk activities, such as clearing land, males had a higher malaria incidence (30.7 [95% CI = 27.6–34.0] episodes per 100 person-years at risk) than females (21.4 [95% CI = 18.7–24.3] episodes per 100 person-years at risk), with a rate ratio of 1.39 (95% CI = 1.17–1.64, $p < .001$ by Fisher's exact test)	<i>Anopheles darlingi</i>	50, 51		
Migrant forest workers	Mekong (Thailand)	Malaria	Overnight stays in the forest carried a higher risk of malaria infection OR = 4.13 (95% CI = 1.29–13.13)		52		
Overnight forest activities, e.g., hunting and travel	Mekong (Lao PDR)	Malaria	Overnight stays in the forest carried a higher risk of malaria infection OR = 2.12 (95% CI = 1.14–3.95)	<i>Anopheles dirus</i>	53		
Collecting food in the forest bamboo, nuts, berries, game animals, and birds	Vietnam	Malaria	Forest work carried a higher risk of malaria infection OR = 2.86 (95% CI = 1.62–5.07) in men but not women OR = 0.71 (95% CI = 0.59–0.86)			Only 2.3% of the population used malaria prevention methods as they cannot afford them. Even after adjusting for the effect of forest work, ethnic group, age, and education, women were still significantly less at risk of malaria. Compared to men, women usually remain well covered, particularly when working outside, thus reducing the risk of exposure to mosquito bites	54

Rubber tapping	Mekong (Thailand)	Malaria	In an area where LLINs and IRS are applied, those earning daily income by performing labor activities mostly in agriculture such as rubber tapping and rubber sheet processing at the smallholdings of rubber plantations were at high risk of malaria OR = 2.92 (95% CI = 1.14–7.44)	<i>Anopheles dirus</i> , <i>Anopheles maculatus</i> , <i>Anopheles minimus</i>	55	
Orchards in tropical forested areas	Mekong (Thailand)	Malaria	Up to 30% acquired orchards planted on former forested areas	<i>Anopheles dirus</i> , <i>Anopheles minimus</i>	56	Use of personal protection such as repellents and permethrin-treated long clothing if working at dawn or dusk
Organized gold and copper mining	Sumatra (Indonesia)	Malaria	90% Of imported malaria between 2009 and 2012 in Sukumbumi health centers (West Java) was among miners from Sumatra who worked night shifts in mines		57, 58	Provision of permethrin-treated work wear by companies for those on night shift recommended. Use of permethrin-treated uniforms did not prevent malaria among soldiers in Thailand, although the design of the study may have influenced the results
Organized open pit gold mining	Iduapriem, Obuasi, Ghana; Siguiri, Guinea; Sadiola/Yatela, Mali; and Geita, Tanzania	Malaria	2010 Malaria incidence per 100 employees Iduapriem 104.62, Obuasi 19.4, Siguiri 22.74, Sadiola/Yatela 9.04, Geita 6.68 despite US\$2 million annual investment in control at the sites in LLINs, IRS, and health education	<i>Anopheles gambiae</i> , <i>Anopheles funestus</i> , <i>Anopheles arabiensis</i>	60, 61	Permethrin-treated work wear for night shift workers. Insecticide-treated clothing prevented malaria by 70% OR = 0.31 (95% CI not reported) in Kenya with <i>Anopheles gambiae</i> , <i>Anopheles funestus</i> , <i>Anopheles arabiensis</i> as the primary vectors

(continued)

Table 7.2 Human Behavior That Necessitate the Use of Complementary Control Tools (continued)

Activity	Region	Disease	Increased Risk of Disease	Vector	Reference	Prevention Strategy	Reference
Military	The Netherlands	Lyme disease		<i>Ixodes ricinus</i>		Use of protective clothing and boots reduced the risk of lyme disease in Dutch soldiers based outdoors to that of the control group based indoors	63
	New Guinea	Scrub typhus		<i>Trombicula</i> spp.		Field tests with dibutyl phthalate applied every 2 weeks to uniforms of Australian soldiers resulted in a 60% and 70% decrease in scrub typhus when it was given to two brigades	64
	South Pacific	Scrub typhus				Uniforms were sprayed with dimethyl phthalate or an emulsion formulation of dimethyl phthalate with an untreated control. All of the soldiers then performed combat operations for 7–10 days in areas with scrub typhus transmission. The dimethyl phthalate spray reduced the number of cases by 64% (from 45 cases in the control group to 16 cases in the sprayed group), and the emulsion reduced the number of cases by 94% (to 7 cases)	65
	Haiti	Dengue	16/241 Italian Army troops	<i>Stegomyia (Aedes) aegypti</i>		Skin repellents protective OR = 0.16 (95% CI = 0.05–0.56), permethrin-treated uniform protective OR = 0.35 (95% CI = 0.11–1.17)	66

67	<p>Although 93 (93.0%) of all febrile patients reported insect bites, only 18 (18.2%) and 40 (40.4%) always used a topical insect repellent and a bed net, respectively. Few had used permethrin to treat the bed net (30.3%) or uniform (13.1%)</p>					
46	<p>The soldiers with treated uniforms exposed in an area with infected sandflies for 6.6 weeks had 83% less leishmaniasis (4 cases out of 143 soldiers) compared with soldiers with untreated uniforms (18 cases out of 143 soldiers)</p>					
69	<p>The attack rate (probable immunes were disregarded from the data) among users was 2/77 and among controls was 9/83 = 0.24, which is a 76% reduction</p>					
70	<p>Insecticide-treated clothing was protective OR = 0.47 (95% CI = 0.20–1.05). Use of no personal protection increased the risk of malaria OR = 2.20 (95% CI = 0.79–6.17)</p>					
30/406 U.S. Army troops	<p>CL <i>Leishmania panamensis</i></p>	Columbia				
	<p>The greatest outbreak of CL occurred between 2005 and 2009, with more than 35,000 cases in the military forces, 80% caused by <i>Leishmania braziliensis</i> and 20% caused by <i>Leishmania panamensis</i></p>					
	<p>Sandfly fever</p>	Egypt				
	<p>93 Cases among deployed U.K. troops within 1 month</p>	Sierra Leone				
	<p>Evangelic and Catholic revivalist sects gather outdoors every evening for hymn singing late into the night</p>	Venezuela				
Religious gatherings	<p>Malaria</p>					
	<p><i>Anopheles gambiae</i></p>					
	<p><i>Anopheles albiparvus</i>, <i>Anopheles oswaldi</i>, <i>Anopheles nunetovari</i>, <i>Anopheles triannulatus</i></p>					

(continued)

Table 7.2 Human Behavior That Necessitate the Use of Complementary Control Tools (continued)

Activity	Region	Disease	Increased Risk of Disease	Vector	Reference	Prevention Strategy	Reference
Missionaries	Haiti	Dengue	After returning from a 1-week missionary trip to Haiti, DENV infection was confirmed in seven (25%). None practiced correct vector bite prevention strategies	<i>Stegomyia (Aedes) aegypti</i>	72		
Workers in the parks and forestry division	North America	Lyme disease	6.3% Seroprevalence among forestry workers and the odds of a recalled tick bite were five times higher among outdoor workers	<i>I. dammini</i>		Those who reported that they always used a repellent had a twofold lower seropositivity for Lyme disease	73
	Poland	Lyme disease	In Poland in 2009, 664 /10,333 (6.4%) cases were certified as occupational exposure among forest workers	<i>I. ricinus</i>	74	The use of permethrin-treated work wear reduces the probability of tick bites by 93%	75
Hiking	North America (Appalachian Trail)	Lyme disease	4% Of long-distance hikers contracted vector-borne disease—principally Lyme disease	Not mentioned, but most likely <i>I. dammini</i>	76	Subjects wearing treated summer-weight outfits (sneakers, socks, shorts, and T-shirts) were 3.36 times (OR = 3.36 with a 95% CI = [2.499, 4.526]) less likely to have nymphal <i>I. scapularis</i> attach to their body than subjects wearing untreated clothing. The odds of nymphal attachment, where ticks were applied below the waist on the leg to shoes, were 74 times less (OR = 73.60, 95% CI = [2.4, 551.45]) for the permethrin-treated group than the untreated group	77

Downloaded by [sarah Moore] at 23:40 08 December 2014

Outdoor recreation—walking, camping, and hunting	North America (northwest California)	Lyme disease, human granulocytic ehrlichiosis	Number of nymphs attaching from sitting on logs: 1.44 per hour; gathering wood: 0.42 per hour, sitting against trees: 0.52 per hour, walking: 1.4 per hour, stirring and sitting on litter: 0.32 per hour, sitting on leaf litter: 0.24 per hour	<i>I. pacificus</i>	78
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Table 7.3 Summary of Repellent Trials

Spatially Active Volatile Pyrethroids						
Trial	Intervention	EPI Effect Size (OR)	VEC Effect Size	Primary Vector	Vector Feeding Behavior	Other Points
79	4 x 0.00975 Metofluthrin coils per house per night	0.39 (0.24–0.62)	32.9% Reduction in mosquito landings by human landing catch (HLC)	<i>Anopheles sudaicus</i>	33% of biting before 10 PM ⁸⁰	Nightly
81	2 x 0.03% Transfluthrin coils per house per night	0.22 (0.13–0.39)	88% Reduction in indoor mosquito densities by CDC light trap	<i>Anopheles sinensis</i>	47% of biting before 10 PM ⁸²	>90%
Topical Repellents						
83	15% Deet lotion in addition to PermaNet 2.0 LLINs	0.94 (0.59–1.48)	98.9% Protection for 5 hours in field tests	<i>Anopheles dirus</i> , <i>Anopheles minimus</i> , and <i>Anopheles maculatus</i>	20%–50% of biting before 10 PM ⁸⁴	About 50%
85	Buzz Off repellent plus PermaNet LLIN	1.16 (0.75–1.80)	>80% Effective against <i>Anopheles gambiae</i> for 8 hours in laboratory tests	<i>Anopheles arabiensis</i>	70% Before 10 PM ⁸⁶	Repellent arm had more malaria to begin with. Effect size calculated by study accounting for imbalance was 0.57 (0.35–0.94), $p = .028$
45	30% PMD lotion in addition to 25 mg/m ² deltamethrin-impregnated bed net	0.05 (0.01–0.20)	Repellent provided 97% protection from <i>Anopheles darlingi</i> for 4 hours ⁸⁷	<i>Anopheles darlingi</i>	48% of biting before 9 PM ⁸⁸	>90% (Per protocol analysis)
89	Repellent lotion containing 20% deet and <i>thunaka</i> (<i>Limonia acidissima</i>)	0.72 (0.50–1.05)	Repellent provided 65% reduction in exposure to <i>Anopheles minimus</i> and 85% reduction in exposure to <i>Anopheles maculatus</i> ⁹⁰	<i>Anopheles minimus</i> and <i>Anopheles maculatus</i>	<i>Anopheles minimus</i> 22% and <i>Anopheles maculatus</i> 62% before bedtime	Compliance actively detected at 84.6%

91	15% Deet lotion in addition to Olyset LLINs	0.89 (0.69–1.13)	Repellent prevented >80% bites from <i>Anopheles arabiensis</i> over 4 hours	<i>Anopheles arabiensis</i>	30% before 10 PM	>90% (per protocol analysis) but application not adequately measured
92	20% Deet and 0.5% permethrin soap	0.42 (0.25–0.69)	<i>Anopheles stephensi</i> and <i>Anopheles culicifacies</i> density—repellent prevented 100% bites over the whole night	100% effective	80% of anopheline biting before midnight	Self-reported compliance >95%
Permethrin-Treated Clothing						
59	Treated uniform with 2 g permethrin per uniform once every 6 months	0.96 (0.71–1.29)	100% effective for 3 months, 84.45% effective up to 6 months	<i>Anopheles dirus</i>	Not measured	100% compliance although it is not known if the uniforms were worn “correctly”
62	Clothing treated with 0.37% permethrin—retreated every 3 weeks	0.56 (0.36–0.86)	41% reduction in blood-fed mosquitoes in users' houses and 41% increase in fed mosquitoes in nonusers' houses	<i>Anopheles arabiensis</i>	Not measured	Not mentioned (assume all clothes were treated) Reported odds of malaria in treatment group is 0.314 $p = .0002$
93	1 g/m ² permethrin-treated chaddars	0.55 (0.38–0.78)	Reduced feeding success of <i>Anopheles nigerrimus</i> , <i>Anopheles stephensi</i> , and <i>Anopheles subpictus</i> by 0%–60%	<i>Anopheles stephensi</i>	80% of anopheline biting before midnight	Not measured
46	Treated uniform with 600–712 mg/m ² permethrin	0.24 (0.07–0.87)	Not measured	<i>Anopheles darlingi</i>	Not measured	Not measured

BOX 7.1 VECTORIAL CAPACITY

$$C = \frac{ma^2bp^n}{-\ln p}$$

- C* = new infections disseminated per person per day by each mosquito
m = number of mosquitoes per person
a = probability a vector feeds on a host /day i.e the proportion of females feeding on man divided by the duration of the gonotrophic cycle in days
ma = the number of bites/man/day
p = probability of daily vector survival
 $1/-\ln p$ = duration of the vector's life in days once it has survived the intrinsic incubation period
n = duration of the extrinsic incubation period in days
b = proportion of sporozoite positive mosquitoes that are infectious

understand the transmission of other vector-borne diseases, including dengue,³⁶ bluetongue,³⁷ onchocerciasis,^{38,39} bancroftian filariasis,^{40,41} and schistosomiasis.⁴² VC describes the potential intensity of transmission by mosquitoes as a function of the (1) human-biting rate, representing the incidence of biting contact between the mosquito and humans in terms of the number of bites per person per day and indicating the number of vector females that could become infected per case per day; (2) expectation of infected life, which is days of infective life per mosquito infected with the given parasite species; and (3) human-biting habit, which is bites on a person per day by an individual female mosquito,³⁵ all of which can be measured using standard field collection techniques.⁹⁴ This exceedingly elegant means of considering the process and impact of vector control on human–vector contact and mosquito survival has been verified with field data³⁵ and provides a convenient logical framework to consider the impact of new vector controls. The majority of work involving the VC equation has considered insecticides that reduce both numbers and life expectancy of mosquitoes and have an excellent impact on reducing malaria intensity. However, in the original article in which VC was described, the author showed that by reducing the human-biting rate by 50% there was a consequent 75% reduction in the VC of the mosquito population.³⁵ VC is extremely sensitive to changes in the biting rate because a vector needs to bite twice to obtain and then transmit a pathogen—hence, human biting is squared in the equation (Ma^2). Thus, the use of repellents will have a strong effect on overall VC by reducing the probability of infecting or being infected by a vector, as described by Ma^2 . Thus, when considering disease control we will define repellents as those interventions that reduce human–vector contact without killing a large proportion of the vector population, that is, those interventions that keep the human population and the vector population apart.

RANDOMIZED CONTROLLED TRIALS FOR MEASURING THE DISEASE IMPACT OF REPELLENTS

Different kinds of evaluations have been conducted to determine the effect of repellents on disease incidence. Randomized controlled trials (RCTs) are currently considered to be the gold standard for testing the effectiveness of interventions for disease reduction in a population,⁹⁵ provided that they are well conducted.⁹⁶ The most important feature of an RCT is that the individuals recruited into the trial are randomly assigned to the intervention or a control, thereby minimizing selection and allocation bias to control as much as possible for both known and unknown confounders that

could influence the correct measurement of impact of the intervention.⁹⁷ Other advantages of a well-conducted RCT are that it facilitates blinding of treatments from investigators, participants, and assessors to prevent bias in the estimation of intervention effect.⁹⁸ It allows for the use of probability theory that any difference seen between the different arms outside the treatment effect is due to chance. A large body of guidance is available to researchers on the importance of correct trial design,⁹⁹ implementation,^{100,101} and reporting.¹⁰²

The main disadvantage of RCTs is the limitation of external validity, that is, the results of an RCT may not be applicable to the general population, due to differences in geographical location, characteristics of the patients recruited, trial procedures, and methods of measuring the outcomes in the trial. For this reason, it is advised that standard methods to ensure quality and reporting guidelines are followed that will allow systematic review and meta-analysis, which aims to collate and synthesize data from multiple studies that meet prespecified eligibility criteria using methods that attempt to minimize bias.⁹⁹ The other disadvantages are cost and time. RCTs are quite expensive¹⁰³ and take several years until the results are published; thus, they may be less relevant at the time of publication.¹⁰⁴ However, when considering the public health implementation of a new vector control product the investment in an RCT is small when considering the importance of implementing a proven intervention that will save lives rather than wasting money on implementing an ineffective intervention (Christian Lengeler, pers. comm.). The cost of the series of RCTs used to generate evidence that bed nets prevented malaria¹⁰⁵ was less than \$10 million; but between 2004 and 2010, \$17 billion was spent on bed nets.¹⁰⁶

Randomized Controlled Trials of Topical Repellents

Southeast Asia

In a refugee settlement in Pakistan, a household randomized trial of Mosbar (a soap containing 20% deet and 0.5% permethrin, which was lathered on but not rinsed off) versus a placebo lotion demonstrated a 56% reduction in *P. falciparum* malaria with an odds ratio (OR) of 0.44 (95% confidence interval [CI] = 0.25–0.76, $p = .004$) and a nonsignificant effect on *P. vivax* malaria with an OR of 1.29 (95% CI = 0.86–1.94, $p = .226$).⁹² The study was carried out on a waterlogged land endemic for malaria, and transmission was effected by *Anopheles culicifacies*, *Anopheles stephensi*, *Anopheles nigerrimus*, and *Anopheles pulcherrimus*, which are predominantly early evening biting vectors.⁹² This characteristic makes topical repellent use ideal as it is applied in the early evening, coinciding with the peak activity of these vectors. This local vector bionomic may have meant that the repellent reduced a substantial amount of malaria transmission and demonstrated the importance of studying the local vector bionomics to determine if the proposed intervention will have any impact on the vector population. The study used simple randomization to allocate treatment to the participants. Randomization minimized the allocation bias of the treatments and confounding factors that were not taken into account. Passive case detection of malaria cases was used, which might have led to the loss of cases that were not reported to the health clinic. Compliance was established by self-reporting of use every fortnight and therefore could not be conclusively ascertained. Field staff, laboratory technicians, and participants were blinded to the intervention. Although this study demonstrated an effect of repellents, it did not take into account the whole malaria transmission season. This study took place for only 6 months, during the *P. falciparum* transmission season and, therefore, demonstrated an effect only against *P. falciparum* malaria. No effect was shown against *P. vivax* malaria because the study was carried out when the transmission of *P. vivax* malaria was low and there were not enough cases to demonstrate a treatment effect. This study would have been stronger if it had been carried out longer to take into account both *P. falciparum* and *P. vivax* malaria transmission seasons. As *P. vivax* malaria is known to recrudescence, the study investigators should have cleared all malaria

cases through an appropriate treatment regimen after checking for individuals deficient in glucose-6-phosphate dehydrogenase (G6PD)¹⁰⁷ so that any cases that were observed would be classified as new malaria cases and not recurrent *P. vivax* cases. Thus, the investigators would have avoided losing malaria cases that they classified as recrudescing cases while they were actually new cases, which reduced the power of the study. It would also have been prudent if the investigators had used active case detection, where they visited all households recruited into the study and screened for malaria, instead of waiting for study participants to report to the camp's health facility. Thus, the investigators would have captured malaria cases of those individuals who visited alternative health facilities or chose to buy drugs directly from drugstores. Active case detection would have also allowed the inclusion of individuals who were too weak to visit the health facility for treatment or found the facility to be too far to seek services. Compliance could also have been better established by conducting frequent spot checks to determine if the study participants did indeed use the treatments they were issued.

In a refugee camp in Thailand, a double-blind randomized clinical trial on the effect of deet mixed with *thanaka* (a root paste made from pulp of the wood apple tree, *Limonia acidissima*, used locally as a cosmetic) compared to *thanaka* alone in pregnant women demonstrated a 28% reduction in malaria incidence, 10.6% (95% CI: 7.5%–13.5%) in women who used *thanaka* and deet, compared to the ones who used *thanaka* alone 14.8% (95% CI: 9.9%–19.7%) in *P. falciparum* malaria, although the difference was not statistically significant.⁸⁹ There was also no significant difference in the transmission of *P. vivax* malaria between the two treatment arms. The lack of a treatment effect was most likely because of malaria transmission being too low to demonstrate a treatment effect as a result of effective and timely diagnosis and treatment of malaria in the camp. As women who were parasitemic during the study were more likely to be anemic on admission than women who had no documented malaria, the authors concluded that they were probably infected before the start of the study, although randomization was performed correctly because anemia was similar between those allocated to treatment and those allocated to control. By treating all the malaria cases before the start of the study so that all cases seen were contracted during the study period, may have reduced prior infection status to bias results, although this would have required a larger sample size and longer study period to observe any treatment effect. The study used both active and passive case detections, which were well correlated. This demonstrates that among individuals with lower immunity to malaria and thus more likely to suffer symptoms, and where malaria screening and treatment is accessible, free passive case detection may be closely related to the actual malaria burden existing in the community and this method can be used as an effective malaria surveillance tool. However, under other conditions, for example, where there are nonsymptomatic malaria carriers or health care is of low quality or is costly to the user, this may not be case. The principal vectors in this area are *Anopheles maculatus* and *Anopheles minimus*, vectors that exhibit a tendency to bite in the early evenings.⁹⁰ This vector behavior demonstrates a circumstance in which repellent use is beneficial, and the fact that no treatment effect was observed suggests that the sample size used was too small to observe the treatment effect or that it may have been useful to use a higher concentration than 20% deet to increase the duration of nightly protection. However, the major finding of the study was that there was no difference in the proportion of congenital abnormalities following the use of deet between treatment and control arms. Also, no deet was detected in the umbilical cord of 46 of 50 samples that were analyzed and none of the 30 samples of urine analyzed were found to contain more deet than the acceptable levels of 0.1 µg/mL. This study reaffirms that deet is safe to use in the second and third trimesters of pregnancy.⁸⁹

In another household randomized, double-blinded placebo-controlled trial recently conducted in Lao-PDR, to determine the effect of 15% deet lotion topical repellent in addition to use of PermaNet 2.0 LLINs on incidence of malaria did not demonstrate any intervention effect.⁸³ Field trials of 10%–20% deet that were carried out demonstrated a 94% protection against all mosquito

bites. The major malaria vectors in this region are the *Anopheles dirus* complex and *A. minimus*, which are both outdoor and early evening biting vectors in the area,⁸² a characteristic that made the repellent an ideal tool for controlling malaria transmission in this setting. However, although the repellent was well received with over 90% of participants reporting that they liked using the lotions, compliance was still low with fewer than 60% of the participants using the lotions more than 90% of the time. Focus group discussions revealed that the assumption that local populations were protected from night biting if they were provided with LLINs was not always true. Adult men and children reported spending time outdoors at night hunting and fishing; they may have benefited from using a longer lasting repellent or even permethrin-treated clothing when engaging in nighttime outdoor activities. These behavioral factors, no doubt, increased bias and reduced the power of the study to detect an effect, if any. The treatment and placebo lotions both smelt and felt the same when applied on skin and were presented in identical bottles identifiable only by a three-digit numerical code. Households were randomized to the treatments by drawing straws labeled with the codes of either the repellent or the placebo lotion. Follow-up visits were done on random dates to ascertain compliance, and the field staff, data entry clerks, and participants were blinded. However, it may have been possible for the participants to distinguish between the two treatments because placebo users were more likely to experience mosquito bites. Treatments were administered at the household level and to no more than 25% of households in any one village. This minimized the chances of treatment contamination, through diversion of mosquitoes from repellent to placebo users, and confusion of treatments, if individuals in the same household were issued different treatments. This might have led to treatment contamination, which can occur through treatment nonadherence (not using the recommended intervention because of perceived lack of effect) and treatment crossover (receiving the intervention intended for the other group in a trial, e.g., repellent users might give or sell their repellent to a neighbor). Both of these scenarios are common in repellent trials and create bias, resulting in an underestimation or overestimation of the treatment effect in either arm of the study. In future trials, this shortcoming can be addressed by using clusters of participants that do not interact with each other, for example, use of villages that are far apart to minimize the chances of participants interacting with each other.

A study carried out in a forest fringe in India to determine the effect of 12% deet used in conjunction with insecticide-treated mosquito nets (ITNs) on malaria incidence demonstrated a threefold (OR = 3.63, 95% CI = 2.27–5.79, $p < .001$) and a fivefold (OR = 5.14, 95% CI = 2.78–9.78, $p < .001$) protective efficacy of the intervention in the first and second years of the study, respectively, when compared to the control arm.¹⁰⁸ This study demonstrated a substantial effect of the use of mosquito repellents and ITNs against malaria. The major malaria vectors in this area are *A. dirus*, *Anopheles philippinensis*, and *A. minimus* which are generally early evening biting vectors¹⁰⁹ where the repellents would protect against early evening biting which may explain why the repellents were additionally effective in reducing malaria among users of ITNs compared to ITN-only users. The ITNs may confer communal protection by reducing vector populations,¹¹⁰ with additional protection from repellent use. This integrated vector management (IVM) using different tools (repellents and ITNs) would therefore have reduced vector populations and host parasite reservoirs by reducing human–vector contact, thereby lowering malaria transmission in the community. The study investigators collected baseline data on malaria incidence and vector bionomics before implementation of the intervention and were therefore able to establish the correct baseline incidence, reducing the chances of underpowering the study by using a smaller sample size. The study was also carried out for 2 years after 1 year of baseline data collection. This increased the sample size of the study, further minimizing the chances of underpowering the study. The study had several positive features: it used active case detection, minimizing the chances of missing malaria cases in the community and making the estimation of treatment effect more robust. The research team also conducted random sniff checks to

ascertain compliance of use of mosquito repellents and ITNs. Another aspect of this study that might have led to such a big treatment effect being observed was the promotion of interventions through information, education, and communication (IEC). For an intervention to be effective, it has to be acceptable by the community. Unlike other repellent studies, this study used IEC, which motivated the community to take up the intervention. This approach demonstrated that repellents can be an effective malaria control strategy if the community is well informed and educated and the intervention is made available. Another finding of significance of this study is the further reduction of malaria incidence in the second year compared to the first year. This demonstrates that continuous implementation of an effective IVM tool can have a great impact on malaria transmission. However, the major shortcoming of this study was the paucity of information on how the findings were analyzed. This omission makes the findings questionable and surprising that the article was published owing to the lack of information on even what method was used to analyze the data, the lack of data on slide positivity rates for the second and third years of the study, and the highly questionable reliance on a converse interpretation of the risk ratio that was presented in the publication. The authors should have provided (1) raw data on the number of cases per 100 man-years per cluster or positivity rates in the first and second years, (2) information on which model was used to analyze the findings, (3) the reason why this model was preferred over other models, (4) information on how the data were interpreted, and (5) information on how bias was accounted for to make the findings credible to readers without having to rely on the interpretation of the authors. The study as presented could not be used in a systematic review.

South America

A household-randomized, double-blind placebo-controlled clinical trial was conducted in Bolivia among the users of a freshly impregnated ITN (25 mg/m² deltamethrin) plus either the insect repellent (*Corymbia maculata citriodon*) with a PMD concentration of 30% (MASTA, United Kingdom) for the treatment group or 0.1% clove oil for the placebo group.⁴⁵ The study demonstrated an 80% reduction incidence rate ratio (IRR) (0.2) (95% CI = 0.11–0.38, $p < 0.001$) in *P. vivax* malaria. However, the effect on *P. falciparum* malaria was not significant most likely due to a lack of power as the number of *P. falciparum* cases was too low to demonstrate any treatment effect. This might be because of an unexpected round of fogging as explained by the authors, but they also offer the more likely explanation that the study took place when transmission of *P. falciparum* was low. Sequential randomization of households was used to allocate treatments, and both the participants and field staff were blinded. Both these attributes increased the robustness of the study, as there was minimal chance of selection bias by the field staff or the participants not using the placebo. The use of a clove oil repellent was useful in this circumstance as both PMD and clove oil have a strong odor, which would suggest to the users that both were active repellents. However, there was always the chance of the control group realizing that they were issued with the placebo as the trial went on and dropping out of the study, thereby reducing its power because of decreased sample size. The study took place for only 4 months, and thus the effect of repellent over the whole malaria transmission period could not be determined. If the study had been conducted for longer to take into account the whole transmission season, then a treatment effect is more likely to have been observed against *P. falciparum* malaria or even a larger, more robust estimate of treatment effect observed as the sample size would have been larger, consequently reducing sampling error and improving effect estimates. The major vector found in this region *Anopheles darlingi* has a peak biting time from 8 to 10 PM⁸⁸ and is strongly exophagic and exophilic;¹¹¹ therefore, it is recommended that repellents be used at this time as people are not under their LLINs. The PMD is extremely effective against even high densities of local malaria vectors and is likely to have provided users relief from high densities

of mosquitoes during the wet season.⁸⁷ Overall, the study demonstrated that the use of mosquito repellents in the early evening in conjunction with LLINs in regions of early evening vector biting did have an impact on malaria incidence, strengthening the case for employment of IVM in malaria control. The compliance of the study participants was reported to be very high, underlined by their preference for PMD measured by focus groups,¹¹² and this was confirmed by random sniff checks by the field staff. The large treatment effect was likely a combination of a well designed and implemented trial methodology conducted in an area where vector bionomics precluded control by other means and where the repellent was well complied with because it was both highly effective against mosquitoes, and cosmetically acceptable to the local population using it.

Sub-Saharan Africa

In a cluster RCT conducted in Ethiopia to determine the effect of Buzz Off repellent on malaria, the odds of contracting malaria was reduced by 43% (OR = 0.57, 95% CI = 0.35–0.94, $p = .028$) for the participants using repellents to supplement PermaNet 2.0 LLINs.⁸⁵ In this study, data were collected by three cross-sectional surveys during the 4-month study. It would have been more prudent for the study investigators to conduct the study throughout the year to take into account the whole malaria transmission season and during the wet and dry seasons. This would have produced a more realistic estimate of malaria in this region. It would also have increased the sample size of the study, thereby decreasing the chances of occurrence of a type II error. Also, some cases of malaria may have been omitted as data were collected for only part of the transmission season. The authors of this trial did not outline the active ingredient and amount present in the repellent. Information on how randomization was conducted was missing; although there was good similarity between socioeconomic variables between the treatment arms, randomization could not have been performed correctly because at baseline the two treatment groups were not similar in terms of malaria prevalence. There was twice as much malaria in the repellent arm of the trial, the control arm complied with and had more LLINs, and two of the eight clusters were sprayed with dichlorodiphenyltrichloroethane (to which arm of the study these were allocated is not stated) and this might have confounded the results of the trial. This resulted in the investigators altering the analysis plan of the study. When the authors followed the analysis plan, outlined in their protocol, there was no difference seen between the treatment arms. As a consequence, the authors changed their analysis, which might have altered the treatment effect observed because the data were not designed to be analyzed in this way.

A double-blind placebo-controlled cluster-randomized trial of 15% deet topical repellent carried out in southwest Tanzania demonstrated a nonsignificant protective effect of 27% reduction in household malaria rates from 91.17 cases per 1000 person-years (95% CI = 198.42–380.76) in the control arm to 65.37 cases per 1000 person-years (95% CI = 110.10–240.84) in the intervention arm ($p = .40$, $z = 0.84$) using the intention-to-treat analysis.⁹¹ These findings were, however, not significant, possibly because the study was underpowered. The major vector is *Anopheles arabiensis*, which bites both indoors and outdoors from 6 pm to 6 am, and it was estimated that a repellent could reduce around 30% of exposure based on the average time to bed of 9 PM. Both semifield and field evaluations of the efficacy of 15% deet repellent demonstrated >90% protection for 4 hours against *A. arabiensis* mosquitoes. However, the effectiveness of an intervention is a component of both efficacy and acceptability by the community of that intervention. Therefore, to ensure effectiveness the study team conducted three rounds of social marketing of the repellent in the study area to encourage usage. This had positive results as usage was reported at 95%. However, despite all these checks that were put in place during project implementation a treatment effect was still not observed. This was mainly due to two reasons: first, the study team overestimated the baseline malaria incidence by extrapolating incidence from all-cause fever data and therefore estimated a

sample size smaller than what was needed to observe a treatment effect. Second, a drought that occurred during the study period lowered malaria transmission such that a treatment effect could not be observed. In future studies, it would be useful to conduct baseline malaria incidence studies to establish correct incidence estimates for sample size calculation. Compliance was determined by self-reporting, which was done at the end of every month when field-workers visited the households to issue new bottles of repellent/placebo. Therefore, compliance in between the visits could not be ascertained. However, random sniff checks were conducted and these spot checks determined that the participants did indeed use the treatments issued. It would, however, have been practical to conduct the checks every fortnight and compare them with self-reported compliance to establish a correlation between the two methods of determining compliance. Passive case detection of malaria by rapid diagnostic tests (RDTs) was used at the local dispensary where participants were offered free diagnosis and treatment. People did not believe the results of negative RDTs and some stopped attending the dispensary, preferring to self-medicate with antimalarial drugs or attend the other health facility in the village that used clinical diagnosis. Also, the health dispensary recruited into the trial may have been sufficiently far from the homes of some participants to prompt them to access alternative health facilities or go to a nearby drugstore. In the future, it would be useful to recruit all health facilities and drugstores in the study area to avoid loss of malaria cases and carry out active case detection. All these factors might have contributed to a reduction in malaria cases, lowering the sample size, thereby underpowering the study. The randomization of interventions and blinding was done as effectively as possible for this case by using treatment and placebo lotions in identical bottles identifiable only by a three-digit code. Even then, as time went by participants realized that they were issued a placebo because they were continuously being bitten. As a result, there was some treatment contamination where placebo users did not use their intervention and repellent users sold their repellents to their neighbors, lowering the power of observing a treatment effect. It was also suspected that study participants gave their identification cards to relatives and friends to benefit from free health care. This would also lead to treatment contamination, which could be overcome with the use of a fingerprint scanner or photographic identification to identify study participants.

A field clinical trial conducted in Isfahan, Iran, to determine the effectiveness of deet sticks against leishmaniasis in 430 students (50% male, 50% female) did not demonstrate any treatment effect.¹¹³ The intervention was reported to be effective for 18–20 hours, and its minimum effective concentration was 55–77 $\mu\text{g}/\text{cm}^2$. Deet placebo was randomized to 330 individuals and placebo stick was randomized to 100 controls, and the treatment allocation code of sticks was revealed only at the end of the study. The children were followed up for 10 months. The efficacy of these sticks was evaluated in terms of the reduction in infection by leishmaniasis using relative risk (RR). Confusingly, in the results section of the study the investigators reported a different number of treatments and controls: out of 200 students who were protected using the placebo pen 2 students acquired leishmaniasis, and out of 230 students who were protected using the deet pen 8 students acquired leishmaniasis. Thus, the study cannot be accurately interpreted.

CASE-CONTROL STUDIES

Apart from RCTs, case-control studies have been conducted to evaluate the impact of repellents on disease. Case controls are observational studies of people with disease and a suitable control group of persons without disease, where a potential risk factor is examined by comparing the frequency of occurrence of the risk factor between these two groups.¹¹⁴ A number of case-control studies have been conducted to determine the effects of repellents on malaria incidence.

In Afghanistan, a case-control study was conducted through social marketing of Mosbar, a repellent soap containing 20% deet and 0.5% permethrin.¹¹⁵ Cases and controls were recruited through passive case detection at a local clinic. The combined use of Mosbar and ITNs demonstrated a 69% reduction in the odds of contracting malaria (OR = 0.31, 95% CI = 0.13–0.72, $p = .007$) compared to control (neither Mosbar nor ITN). The local mosquito vectors *Anopheles stephensi* and *Anopheles nigerrimus* bite shortly after dusk, and throughout the night, a characteristic that makes the repellent a suitable control tool for evening protection before LLINs can be used. The repellent selected was highly efficacious and gave 100% protection for the whole night, which might have promoted the observation of treatment effect. However, as a hospital-based case control this study was prone to selection bias and therefore could not be generalized to the rest of the population, as individuals attending the clinics recruited into the trial might have had different characteristics from individuals in the general population. There are a number of anecdotal case-control studies that were not specially designed to measure the effect of repellents as shown in this study but to identify risk factors among those with malaria.

In a case control study of risk factors among British travellers returning from the Gambia less use of repellents was associated with a greater risk of contracting malaria.¹¹⁶ The use of repellents, applied either on the skin or on clothes, is a key strategy for bite avoidance recommended in travel medicine. This finding illustrates the importance of using repellents when traveling to malaria-endemic regions. Therefore, all individuals traveling to malaria-prone areas should be advised to use malaria control strategies to protect against malaria. Also, tourist destinations should provide information on the vectors that are present in these regions so that the tourist can be better advised and prepared on which tools to use. It also emphasizes the importance of having international guidelines for travelers visiting malaria-endemic regions to avoid importing malaria cases to their mother countries.

In Kilifi, Kenya, in a large (>1500 participants), well-designed case-control study the use of local repellents, mosquito coils, and insecticide sprays was significantly associated with protection from developing severe malaria after adjusting for confounders (OR = 0.57, 95% CI = 0.35–0.94, $p = .02$). The cases and the controls were chosen from the same area in the community. Consequently, the results could also not be generalized to the whole population as the individuals from this area of the community might be different from other members of the community. It would have been better to select more than one study area to make the findings more general to the population.¹¹⁷ A study from Gambia that used a design almost identical to the study in Kenya showed an association with the use of coils in preventing severe malaria in a univariate analysis, but this effect disappeared on multiple logistic regression.¹¹⁸

The overall evidence generated by the aforementioned studies demonstrates that the use of repellents can be effective against malaria transmission if these interventions are used correctly and with sufficient frequency. In studies where an association cannot be established, it is usually because of poor study design. The following series of studies are inconclusive due to a number of factors including poor matching; poor attention to sample size; and poor measurement of compliance, which is the single most important factor in the effectiveness of any repellent.

In another case study in India, individuals who did not use repellents had nonstatistically significant lower odds of malaria, with an OR of 0.85 (95% CI = 0.57–1.28, $p = .41$), compared to those who used repellents. This finding is not consistent with other repellent trials and there are various factors that might have led to this conclusion, especially as those exposed to higher levels of mosquito bites are more likely to use mosquito prevention tools. In addition, the cases and controls were not matched because the controls were recruited from the same clinic, assumed to have come from the same socioeconomic, demographic, and geographical area as the cases. Because of the study design, there was no way to establish compliance to repellent use. Also, the longevity and quality of these repellents could not be established, although the mosquito coils and mats used were reported to be allethrin and the topical repellents used contained diethyltoluamide, for which the concentration was not mentioned. The bionomics of the local vectors was not discussed to determine whether the use of repellents would be an appropriate tool.¹¹⁹

Similarly, in another case-control study in Burkina Faso use of mosquito coils and burning of plant leaves for smoke (spatial repellents) were not associated with a lower risk of malaria, with an OR of 1.24 (95% CI = 0.73–2.00, $p = .47$) and an OR of 0.74 (95% CI = 0.35–1.56, $p = .43$), respectively. Like the aforementioned study, use of mosquito coils and burning of plant leaves for smoke were self-reported. The study participants might have overreported or underreported, biasing the findings on the study. The controls were recruited from the same residential area. As a result, these findings cannot be generalized to the whole population, as the individuals from this area might not have similar characteristics to the general population. The controls were not actively tested for malaria and were assumed to be malaria negative. This might have biased the study toward the null hypothesis if the controls were positive for malaria.¹²⁰

In Ecuador and Peru, a community-randomized trial of Mosbar, a mosquito-repellent soap containing 20% deet and 0.5% permethrin, did not show any significant reduction in malaria incidence between the intervention and control groups.¹²¹ The effect of the repellent soap was studied under different settings. It was found to be efficacious only when individuals wearing the soap were inactive after application. This contrasts with the findings from Pakistan⁹² where the repellent was extremely effective in preventing mosquito bites. The differences observed might be due to the higher relative humidity in the Ecuadorian site that caused more rapid loss of repellent through sweating. Compliance to repellent use was not established and lack of treatment effect may have been due to poor compliance, as many people did not like the smell of the repellent and in Ecuador, because of humidity, a thick layer of soap remained on the skin, which was not pleasing to the users. As compliance requires a high degree of motivation, it was necessary for the study team to socially market their intervention to encourage its use and user acceptability. Interestingly, user compliance was drastically reduced when the soap was only made available from shops and was no longer available free of charge. This was similar to findings in other studies and underscores the importance of developing low-cost or highly subsidized interventions that can be accessed by those of low socioeconomic status in disease-endemic countries who are also those most at risk from disease morbidity and mortality. For an intervention to be effective, it has to be acceptable, affordable, or free to the community.

CROSS-SECTIONAL STUDIES

Cross-sectional studies are research methods that involve observing all of a population or a representative subset at a specific point in time. They collect data on outcomes and/or exposures collected on each participant at one moment in time. Thus, although they are simple and quick to perform, they are more robust at measuring associations with chronic diseases because they measure prevalent rather than incident outcomes. Cross-sectional studies that collect data on both outcome and exposure are not very robust in establishing the causal effect of an intervention, as they are prone to bias from confounding factors, but they can be used to test hypotheses about interventions and to justify a research objective.

A cross-sectional survey was carried out in the Thailand–Myanmar border in Northern Thailand to determine the risk factors that contribute to malaria infection. Malaria prevalence was extremely high in 46% of the participants. It was a well-designed study that had correctly used sample size calculation and demonstrated a clear relationship between working or staying overnight in the forest and having malaria in univariate and multivariate analyses, although the use of topical repellents and long clothing was protective against contracting malaria on univariate analysis, but this treatment effect was not seen when confounders were taken into account. This study shows some of the practical scenarios where topical repellents can be used, like individuals working in the forest or in crop fields who are not able to use conventional control measures like LLINs.¹²⁸

A cross-sectional survey to determine the effect of personal protective measures (PPMs) against malaria in travelers demonstrated a significant reduction in malaria among travelers who used protective clothing covering their arms and legs. However, no significant reduction was associated with the use of repellents and coils. As explained in this study, compliance to PPMs was very poor among a large proportion of the study participants. This would likely explain the lack of treatment effect. Also, it is advisable that more stringent measures by responsible agencies are introduced to ensure compliance to PPMs by people traveling to malaria-endemic regions to avoid the exposure of nonimmune individuals to malaria and also reduce the importing of cases to their mother countries.¹²³ Compliance to personal protection is surprisingly low among those with access to the correct preventive measures. A recent survey among 2205 individuals from the French military during and after a stay in malaria-endemic areas were exposed to malaria incidence of 2.98 cases per 100 subject-years in malaria-endemic areas.¹²⁴ The “correct” compliance rates were 48.6% (95% CI: 46.5%–50.7%; ranging from 2.6% to 88.2%), 50.6% (95% CI: 48.5%–52.7%; ranging from 1.7% to 97.3%), and 18.5% (95% CI: 16.8%–20.1%; ranging from 4.9% to 59.6%) for wearing long clothing at night, using LLINs while sleeping, and using insect repellents, respectively. Factors that often influence compliance are gender, the rainy season, mosquito bite burden, and perceived mosquito attractiveness compared with other people, while perception of the severity of malaria was not associated with regular use of any of the methods measured. A further cross-sectional survey of 89,617 travelers returning from East Africa was conducted between 1988 and 1991.¹²³ Only 2% of respondents stated that they regularly complied with air-conditioned rooms and/or bed nets, adequate clothing, and use of insecticides and/or coils. Regular use of personal protection resulted in a small but significant reduction in malaria incidence when travelers were interviewed 12 weeks after returning home, but each method alone showed no significant effect. Unlike the situation among the French military travelers, the holidaymakers increased their compliance during periods when more mosquito bites were noticed; but similar to the French study, gender had no significant influence on compliance and, surprisingly, neither did diagnosed or suspected pregnancy. Those using no chemoprophylaxis were not more vigilant in preventing mosquito bites. Compliance diminished continuously with the length of stay in Africa: among those who stayed up to 2 weeks the compliance rate was 77.2%, whereas in those staying 2 months or more the rate was 63.3% ($p < .001$).

OUTBREAK REPORTS

In South Africa, topical application of 15% deet to feet and ankles reduced overall *Anopheles arabiensis* bites by 69% in field observations. This led to the testing of this intervention under operational conditions during a malaria outbreak in Mpumalanga, 15 km south of the Kruger National Park. The implementation of the intervention was associated with an immediate drop in malaria incidence from 42 to 10 cases per week. This effect is, however, difficult to interpret as it could have been due to repellent use and it could also have been due to the fact that the epidemic curve had peaked and was dropping naturally. The repellent may, however, have helped in maintaining the low incidence of malaria. But this study does give situations where repellents can be used. The most likely reason why the more effective LLINs were not used in this particular scenario is that the major vector in this area, *A. arabiensis*, had behaviorally adapted to outdoor biting and the secondary vector, *Anopheles funestus*, had developed resistance to IRS.¹²⁵ Although the results are not clear, this study represents a useful scenario in which repellents might be employed against malaria.

In an outbreak report that described the outbreak of *P. vivax* malaria in Far North Queensland, Australia, individuals who used topical repellents (deet) were at 0.01 (95% CI: 0.00–0.19) the odds of developing malaria compared to those not using repellents. The findings of this study reinforce the need to use other PPMs in areas when conventional malaria control tools are not applicable.¹²⁶

During an outbreak in India, a well-designed investigation was conducted where malaria cases were slide-confirmed and compared with matched neighborhood controls. For both groups, information on personal protection use was gathered by questionnaires and data was compared using matched odds ratios (MORs).¹²⁷ In total, 7303 cases and 17 deaths were reported between April 2005 and March 2006 with a peak during the October rains (attack rate: 50 per 1000, case fatality: 0.2%), and half of the cases were detected by active case detection. Use of repellents was associated with an odds ratio of 0.1 (95% CI: 0.06–0.3) of contracting malaria, and failure to use repellents was associated with 69% of malaria cases in the population. Compared with controls, cases were more likely to sleep outdoors (MOR: 3.8, 95% CI: 2.2–6.5) and less likely to use mosquito nets and repellents (MOR: 0.3, 95% CI: 0.1–0.5). In this outbreak investigation, the villagers reported the use of repellents and coils and, therefore, correct and consistent compliance could not be established. This might have biased the treatment effect seen. Also, being a retrospective case control this study might have been prone to recall bias. Despite these shortcomings, this study demonstrated a protective trend of mosquito repellents against malaria.

There are a large number of disease outbreak reports among military personnel related to non-compliance with standard PPMs.¹²⁸ A report from the French Army monitoring leishmaniasis among troops stationed in Guinea showed four separate outbreaks of leishmaniasis in which the troops admitted that they did not use personal protection correctly.¹²⁹ In a malaria outbreak in French Guiana, a retrospective cohort study found that malaria was associated with a low compliance of impregnated battle dress uniforms (BDUs).¹³⁰ This study also shows the problem of compliance to repellent use. As studies mentioned earlier have shown, for repellents to be effective they must be acceptable to the individuals to whom they are issued and must be used correctly and consistently. Similarly, in a malaria outbreak in Sierra Leone among British soldiers a case-control study demonstrated that the use of insecticide-treated clothing offered significant protection against malaria with almost 50% fewer cases being reported among those individuals who used their impregnated BDUs (OR = 0.57, 95% CI = 0.20–1.05, $p = .045$). Interestingly, the use of multiple protection measures gave even better protection (OR = 0.29, 95% CI = 0.10–0.80, $p = 0.007$). However, the use of repellents and chemoprophylaxis showed no significant effect.⁷⁰ In a malaria outbreak in 2003, 44 U.S. Marines were evacuated from Liberia with either confirmed or presumed *P. falciparum* malaria.¹³¹ An outbreak investigation showed that only 19 (45%) used insect repellents, 5 (12%) used permethrin-treated clothing, and none used bed netting, demonstrating further the importance of compliance in personal protection from vector-borne diseases.

PERMETHRIN-TREATED CLOTHING EVALUATION

Randomized Controlled Trials

Southern and Southeast Asia

In Afghanistan, an RCT on 1 g/m² permethrin-impregnated *chaddars* (cloth used as a head covering [and veil and shawl] by Muslim and Hindu women) reduced the odds of having *P. falciparum* and *P. vivax* malaria by 64%, OR = 0.36 (95% CI = 0.20, $p = .001$), and 38%, OR = 0.62 (95% CI = 0.36–1.06, $p = .069$), respectively. There was a significant effect in the 0- to 10-year and 10- to 20-year age groups. This trial, however, showed no effect on malaria incidence in refugees >20 years of age.⁹³ In this study, no information was given on how the randomization was carried out. The trial took place over 5 months and, therefore, did not capture the effect of repellents over the entire malaria transmission season. The study was carried out at the end of the *P. vivax* transmission season and at the start of the *P. falciparum* season; this might explain why

there was a larger treatment effect seen on *P. falciparum* transmission compared to *P. vivax* transmission. It is possible that if the study had been carried out longer, then a larger effect would have been observed. As *P. vivax* malaria is known to recrudescence, the study investigators should have cleared all malaria cases through an appropriate treatment regimen after checking for G6PD-deficient individuals⁵⁷ so that any cases that were observed would be classified as new malaria cases and not as recurrent *P. vivax* cases. The study used passive surveillance of malaria cases; consequently some cases not reporting to the health clinic might have been missed, lowering the sample size and power of the study to observe a treatment effect. This might explain why a treatment effect was not seen among females, because they were less likely to leave their homes due to the practice of *purdah*. In the evening, they might also have been using their *chaddars* as bedding for their children as a protective effect was seen only among those individuals <20 years of age. Compliance was established by visiting the households every 2 months. As frequent compliance inspection was not done compliance in between the months cannot be ascertained, and hence the findings of the study are less robust. As with all intervention studies, compliance is essential for an intervention to be considered effective, although the *chaddar* is a piece of clothing that is used on a daily basis.

A second single-blind RCT by the same group that investigated the effect of ITNs, insecticide-treated *chaddars* used to sleep in, and residual pyrethroid spraying of individual houses for the prevention of cutaneous leishmaniasis (CL) in Kabul, Afghanistan, also demonstrated a significant protective effect.¹³² The incidence of CL among those randomized to the control was 7.2%, among ITN users 2.4% (OR: 0.31, 95% CI: 0.2–0.5), among impregnated *chaddar* users 2.5% (OR: 0.33, 95% CI: 0.2–0.6), and among those living in λ -cyhalothrin-sprayed houses 4.4% (OR: 0.60, 95% CI: 0.3–0.95). ITNs and impregnated *chaddars* were equally effective, providing about 65% protective efficacy, with approximately 40% protective efficacy being attributable to individual house spraying. The study was well powered: it was conducted in 1997–1998 among a nonimmune population of 3666 people over 15 months. New cases of CL were diagnosed based on clinical criteria diagnosed by the inspection of lesions, but parasitological confirmation could not be completed after aid organizations were ejected from Kabul in July 1988. Another difficulty of working in such a challenging environment was that compliance could not be measured, because spot checking would have invaded the privacy customs strongly upheld in the region. No significant differences for age or sex were found between new cases in the intervention and control groups. No serious side effects were reported, and interventions were generally popular; ITNs were the most popular, followed by residual spraying and then impregnated *chaddars*. Both ITNs and *chaddars* are useful in this region, as the population tends to be quite mobile. This population mobility caused massive loss to follow up (45%) as people moved out of the study area, but the study investigators had anticipated this and accounted for it during the recruitment of study participants. This demonstrates the importance of recruiting the appropriate sample size in any study.

A double-blind placebo-controlled trial to determine the efficacy of permethrin-impregnated uniforms among Iranian soldiers in Isfahan demonstrated a reduction in the odds of contracting CL. However, this effect was not significant, possibly because the study had only 134 people per treatment arm for 3 months of exposure in the field (1608 person-weeks per arm). Compliance was high, as the soldiers were required to wear the uniforms day and night and compliance was monitored. As compliance was ascertained, the results of this study may be credible. However, the method used for randomization was not described. This may have been done incorrectly, biasing the study and hence the observation of no treatment effect in the treatment arm. Both the participants and the study investigators were blinded to the treatments, reducing chances of selection bias.¹³³ The study, however, showed that permethrin-impregnated uniforms are safe for human use and no adverse effects were observed. Therefore, they present a potential tool that can be explored for malaria control. The fact that all the lesions (sites of infection) among the treated

uniform group were on sites unprotected by the uniform (face and wrist) is of importance; but in the control group, lesions were found on the arm and trunk. If the soldiers had been using full personal protection including a topical repellent for use on their face and hands,¹³ they may not have contracted leishmaniasis.

In the Thailand–Cambodia border, a randomized placebo-controlled trial evaluating the effect of 2 gm permethrin per treated uniforms versus kerosene-treated uniforms on preventing malaria among the Royal Thai Army demonstrated no effect. The population was 403 male soldiers on active duty for 6 months. The randomization method was not outlined in this study, and compliance could not be established at all times. Both these factors could have confounded the findings of this study as the selected study participants might have had confounding characteristics. Also, as compliance could not be established both groups might not have used the repellent, therefore biasing the study toward the null hypothesis. One study arm may also have not complied with the intervention and similarly driving the effect toward the null.⁵⁹

South America

A double-blinded placebo-controlled study in Colombia among 86 soldiers randomized to 600–712 mg/m² permethrin-treated uniforms and 86 soldiers randomized to water-treated uniforms over 4.2 weeks showed the uniforms to be 79% protective against malaria, 3% versus 14% among treated and control groups, respectively, and 75% protective against CL, 3% versus 12% among treated and control groups, respectively.⁴⁶

The same double-blind RCT carried out in Colombia to determine the efficacy of permethrin-impregnated uniforms against both malaria and CL demonstrated a reduction in the RR of malaria (RR = 0.29, $p = .015$) and CL (RR = 0.21, $p = .002$).⁴⁶ As adherence to instructions to wear the permethrin-treated clothing day and night could not be monitored, the findings of this study are debatable, as with all studies in which compliance could not be established. However, the monitoring of disease was actively done every day and it is unlikely that any cases of malaria or CL could have been missed. There were very few reports on the adverse effects of insecticide-treated clothing. This is similar to other studies where very few adverse effects were reported, reinforcing the proposition that insecticide-impregnated clothing is safe for human use. This intervention can be applied to normal clothing, thereby tackling the problem of adherence so often seen when using topical repellents.

Sub-Saharan Africa

In a randomized community trial among 198 Somali refugees of all ages and both genders with no known allergies or respiratory problems at the Dadaab refugee camp, participants were randomized to either 0.37% permethrin or water placebo used to treated clothing and bedding, retreated every 3 weeks for a period of 3 months. All clothing and bedding was treated, including *diras*, saris, *jalbaabs*, *ma'awis*, shirts, sheets, and blankets. Use of the permethrin-treated clothing and bedding significantly reduced the odds of contracting malaria by 70% (CI was not reported).⁶² Methods for randomizing treatments were described as systematic random sampling of households within treatment and control blocks 1.5 km apart, and compliance was maintained by regular retreatment of all clothing and bedding. The participants and laboratory technicians were blinded to the treatments. These aspects of the design are positive. However, the study was small and the statistical reporting was not good as it was unclear, it was overreliant on models, and p values and ORs were reported without CIs. However, the study reported the percentage positive in the treatment and intervention groups and the number of people tested, so these data could be used for a meta-analysis.

In another randomized community trial in Kenya to determine the effect of appropriate permethrin-impregnated clothing against malaria, it was found that the IRR of contracting malaria in those aged over 5 years in the intervention group was 0.187 (95% CI = 0.046–0.770, $p = .02$) compared to the control group.¹³⁴ For those under 5 years of age, however, no effect was seen. A total of 472 individuals were enrolled in a randomized community trial where the unit of randomization was the hamlet (*manyatta*) with 234 and 238 in the experimental and control arms, respectively. Baseline data included sociodemographic data, parasite prevalence data from thick and thin blood smears, and clinical measures of malaria. The intervention involved the dipping of *shukas* owned by the experimental group in permethrin, although the dose was not available in the publication. The prevalence of malaria in the study population (based on slide confirmation) was considerably lower than that used for the power calculation based on clinical estimates (2.2% vs. 20%). For those aged 6 or over, the rate of malaria cases (events per 10,000 person-days at risk) was 1.41 in the experimental group versus 7.49 in the control group (IRR: 0.187, 95% CI: 0.046–0.770). For children less than 5 years of age, results were imprecise with no clear benefit of the intervention. An attempt was made to impregnate all *shukas* of the experimental group. However, some children refused to have their *shukas* dipped in the cold early morning hours, as it was their only clothing. Other children, one-third of the 5 years and under in both groups, owned no *shuka*. The researchers had been aware of this before the study, but had felt that this would not affect results because preliminary research had indicated that the children without *shukas* slept under their mothers' *shukas* at night. Of the four cases that occurred in the intervention group, three did not own *shukas* and the fourth owned a *shuka* that was not impregnated. This incomplete coverage, coupled with the fact that the study investigators did not establish the local baseline incidence rate, led to an underestimation of the sample size required to observe a treatment effect. This shortcoming underlines the importance of establishing baseline factors before any study is implemented. Clinical reports implied that 35% of all patients were seen for malaria, and the clinicians' predicted prevalence of parasitemia was even higher (50%). Although a more conservative 20% was used to calculate sample size, the 2.2% parasitemia observed at baseline clearly reduced the statistical power of the study. This highlights the unreliability of malaria reports based on clinical diagnoses, which was also one of the reasons for the Tanzanian study of deet repellent being underpowered.

Other Studies

In a clinical trial in Myanmar, the use of treated scarves and hand bands were significantly associated with a lower incidence of malaria compared to the control arm where these interventions were not used.¹³⁵ The major local vector was *Anopheles minimus*, an outdoor and early evening biting mosquito. This makes treated scarves and hand bands appropriate control tools in this setting, as conventional tools cannot be used at these places and times. The study was carried out for a short period of time and did not take into account the low transmission season and was therefore not possible to establish the seasonal effect of this intervention. Compliance assessment was carried out in 10% of the study participants. From this sample, the compliance of the entire study population could be inferred. Also, the investigators carried out regular bimonthly checks on compliance and random spot checks. The compliance monitoring of this study was well conducted, and the results can be considered credible. The results from toxicity evaluations of this intervention did not demonstrate any adverse effect. This was in agreement with other studies that assessed the toxicity of insecticide-treated clothing.

All the earlier mentioned studies are associated with a protective trend of repellents against malaria. Most studies had questionable study designs and, therefore, the results of these studies could not be conclusively relied on. However, the fact that a protective trend was observed

in all of them reinforces the need to conduct a well-designed, large-scale trial to ascertain the effect of repellents on disease incidence.

MOSQUITO COILS

Randomized Controlled Trials in Southeast Asia

There have been two randomized trials evaluating the impact of burning mosquito coils every evening on malaria transmission, both conducted in Southeast Asia. The first study⁸¹ was a single-blind, cluster-randomized comparative control clinical trial conducted in Ruili district, Yunnan province, People's Republic of China, close to the Myanmar border between April and October 2007. Yunnan is one of only two provinces in China that still has malaria cases and the Ruili district has a particularly high number of cases. The area is heavily forested, a high proportion of migrant populations moves over the border between countries, and it has many remotely located minority group habitations, making implementation of vector control and public health programs extremely difficult. All the communities enrolled were in rural areas.

The trial was designed to measure and compare the protection against malaria provided by mosquito coils, LLINs, or a combination of the two. The study recruited 2052 households comprising 7341 individuals, excluding individuals under 6 years and pregnant women. Households were randomized into one of four groups: coils (0.03% transfluthrin coils, SC Johnson), deltamethrin LLINs (TianJin-Yorkool, Ltd., Tianjian, People's Republic of China; and Lantrade Global Supplies, Ltd., Gerrards Cross, United Kingdom), coils plus LLINs, and a control group without any intervention other than whatever control intervention they were already using. At baseline and every month post intervention, each individual was actively screened for malaria (both *P. falciparum* and *P. vivax*) by RDT. At the end of the 6-month study, there were 69 confirmed malaria cases in the control group, 16 in the coil group, 14 in the LLIN group, and 5 in the combined coil plus LLIN group. In the coils-only group, the age-adjusted OR for *P. falciparum* malaria was 0.23 (95% CI = 0.11–0.50, $p = .0002$) and protective efficacy against *P. vivax* was 80%, OR = 0.20 (95% CI = 0.09–0.44, $p < .0001$), and were not significantly different from those for LLINs or LLINs plus coils. The level of compliance with the allocated interventions was high: it was noted that >94% of individuals used coils and/or LLINs for >90% of the month prior to the surveys. Conversely, those in the control arm were less compliant, with 13%–19% using local coils for 3 or more days per month. A per-protocol analysis including only those with >90% compliance gave almost identical results to the intention-to-treat analysis.

A second, more recent double-blind, placebo-controlled cluster-randomized trial conducted in Sumba, Indonesia, to evaluate the effect of 0.0097% metofluthrin mosquito coils only (no LLINs were used in either study arm) against malaria⁷⁹ comprised two clusters (1000 people) allocated to the treatment arm and two clusters (1000 people) allocated to the control arm. Of these, 45 healthy males who were >17 years, >40 kg, G6PD normal, and resident in the village for the study period in two clusters per arm ($n = 90$ per arm) were followed up as the study cohort for 26 weeks. Compliance with mosquito coils was monitored daily and malaria was monitored weekly among participants by active case detection. In addition, malaria vector abundance and biting time was measured by indoor and outdoor human landing catch; vector population age was estimated from parity rates by detinova ovarian dissections, and sporozoite rate in vectors was measured by CSP-ELISA (circumsporozoite protein enzyme-linked immunosorbent assay). Malaria incidence among the treatment group was 0.904 versus 2.324, which equates to a 61.1% protective efficacy (95% CI = 37%–75%, $p < .00001$).

CONCLUSIONS

These two trials^{79, 81} of spatially acting pyrethroids used as mosquito coils were tested in isolation, without the addition of LLINs, and provided unambiguous evidence that individual malaria risk is significantly reduced by >60% simply through avoiding mosquito bites. These trials were conducted under rigorous conditions that should set the benchmark for future trials, because they were designed, powered, and analyzed with the help of a statistician; had adequate randomization; were placebo controlled, allowing adequate blinding¹¹; and used active case detection with RDTs with polymerase chain reaction confirmation throughout the study. In addition, essential to the success of any repellent study, very high compliance was observed throughout, which was carefully monitored by study staff. Furthermore, both studies were conducted in suitable field sites. In both cases, a large proportion of mosquito bites occurred before bedtime (Table 7.1) and mosquito coils were culturally acceptable (a smoky environment is tolerated). Furthermore, repellents may be more effective in Southeast Asia because malaria transmission is low and seasonal and the main malaria vectors are opportunistic and will feed on other hosts.

Future trials should attempt to match the high standards of these trials and also include some further information on community-level measurements of the impact of mosquito coils on malaria vector population dynamics. These data were collected in some extremely detailed studies on dichlorvos during the 1960s and showed that at a high enough coverage of repellent interventions there can be a community protection demonstrated by decreased human–vector contact, vector infectiousness, and vector longevity.

This is the key piece of information that should be collected from any future trials of personal protection tools if they are ever to be considered as public health tools applied at a community scale to prevent disease transmitted outdoors, in the day or evening, rather than just niche tools for particular lifestyles or occupations. Furthermore, dichlorvos is an example of a repellent tool that requires little compliance—it just requires the replacement of dispensers every 2 weeks. It is essential that future research examines such low-compliance interventions that will help to address the two greatest barriers to repellent implementation: cost and compliance.

Findings from the review strongly support the theory that use of repellents has a beneficial protective effect against transmission of disease, mainly, malaria and leishmania as very little data are available on dengue. Even though individual studies had varying outcomes, the combination of all the available evidence does support the notion that specific repellents should be incorporated into current vector control strategies where appropriate. We recommend the use of repellents (both spatial and topical) at times when current control measures cannot be implemented. The other key finding from this review was the paucity of existing high-quality data. To improve the speed at which products are developed and approved by bodies such as the WHO, there is a clear need for harmonization of methodologies and outcomes measured in new trials and evaluation of vector control tools, in particular, the way they are reported. Researchers need to be encouraged to ensure that their piece of research contributes to the overall picture in a research field. Clear reporting of outcomes and use of guidance available for this task, for example, using CONSORT guidelines,¹³⁶ should make future trials more robust and data easier to assimilate by means such as systematic review and meta-analysis for use by policy makers. It was also clear from this review that those trials collecting data through active case detection were far more powerful than those using passive case detection. Important secondary end points of any trial are entomological correlates of reduced infection, that is, human–vector contact, parity rate, sporozoite rate through regular human landing catches, and human compliance with the intervention. An exposure-free measurement of human landing is especially needed for large-scale epidemiological work particularly in areas where dengue or other arboviruses are prevalent. Measurements of compliance such as salivary antigen markers of exposure to mosquito bites¹³⁷ are a key research need for rigorous and ethical research into disease prevention using vector control tools as the markers of exposure may be used as a measure of both exposure and compliance.

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