London School of Hygiene & Tropical Medicine

A cluster-randomised trial to assess whether the insect repellent *N,N*-diethyl-*m*-toluamide (DEET) can provide additional protection against clinical malaria over current best practice in Lao PDR.

Submitted for the degree of PhD

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I, Vanessa Chen-Hussey, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Background: Malaria remains a serious threat in the Greater Mekong Sub-region (GMS), not just from the direct impact on human health, but also from the emergence and spread of resistance to artemisinin, the last remaining effective antimalarial. Malaria control in this region is therefore a high priority on a global as well as local scale. In the southern region of the Lao People's Democratic Republic (Lao PDR) as across much of the GMS malaria vectors are found biting outdoors in the early evening before people are protected by long-lasting insecticidal nets (LLINs). Therefore there is a need for additional malaria control tools that can protect people during these evening hours.

Methods: Human landing catches in a village setting in southern Lao PDR were used to evaluate the protection from evening biting given by repellent lotions containing 10-20% *N,N*-diethyl-*m*-toluamide (DEET). A randomised controlled trial was designed to test the effect of 15% DEET against malaria. A total of 1,597 households were recruited and randomised to either the repellent or a placebo lotion. All households were also provided with LLINs. The acceptance and compliance with the repellent lotion was assessed through exit questionnaires and focus group discussions (FGDs). A meta-analysis was then carried out to put the results from the Lao PDR in context with other repellent trials.

Findings: All DEET concentrations provided at least 96% protection from evening biting over five hours. However 15% DEET was determined to be the best choice of intervention over 10% DEET after also considering the results of other similar trials. Intention to treat analysis of the randomised controlled trial found no difference between treatment arms after accounting for gender and socio-economic status (incidence rate ratio 0.96, 95% confidence interval 0.54-1.71, p=0.886). According to protocol analyses of participants who used the lotions over 90% of the time also found no effect from repellent use after other factors had been taken into account (incidence rate ratio 1.45, 95% confidence interval 0.53-3.99, p=0.467). The most

important predictor of malaria incidence was socio-economic score which indicated that lower wealth was significantly associated with an increased malaria risk. 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. It emerged from FGDs 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. The protection from malaria by repellent use in this trial was lower than in other randomised controlled trials carried out in Bolivia, Pakistan and Tanzania. The meta-analysis found that repellent use was associated with a 33% reduction in *P. falciparum* incidence (95% CI 0.42-1.09, p=0.11) and a 35% reduction in *P. vivax* incidence (95% CI 0.18-2.34, p=0.51), however neither figure reach significance.

Interpretation: Limitations of this trial include the compliance level which was lower than in other trials. In addition the variability inherent in topical repellents may make them unsuitable for use as an intervention. The outcome of this trial shows that topical insect repellent is not a suitable wide-scale intervention against malaria and does not provide significant protection over and above LLINs in an area of outdoor biting. However, repellents do undoubtedly reduce biting and therefore their potential to be effective intervention tools remains. Future work should concentrate on forms of repellent that can be better standardised such as impregnated clothing. If successful then further research into mosquito response to repellent is recommended including, where best to apply and the potential for the development of resistance.

Acknowledgements

This project would not have been possible without the late Dr Nigel Hill. His tireless support and much needed advice during my first year were invaluable. His good sense and more importantly great sense of humour are greatly missed.

A huge thank you goes to Prof. Steve Lindsay for his inspiration, enthusiasm and of course literary criticism. It has been a privilege to have such a gifted teacher as my supervisor once again. Thank you for your support over the past two years and for taking me on after Nigel's death.

I am also indebted to Ilona Carneiro for her advice on all things statistical throughout the design and analysis of the trial. Thanks also to Jane Bruce for her last minute support.

Thanks to Terri O'Halloran for being generally amazing and also for her efforts to fix some very broken paperwork.

There are numerous people in Laos I would like to thank for contributing their time and energy to this project. In particular the only other person who worked full time on the trial, Santi Sayarath, who taught me a both Lao and logistics during the year we worked together. Dr Hongkham from the Lao Ministry of Health's Centre for Malariology, Parasitology and Entomology helped immensely during field trips and was always ready with a calm word and a cheerful smile. Rob Gray, the director of PSI Laos, provided steady guidance and support along with the rest of the PSI staff. Special thanks go to Siha, Oi, Saysana, Magnoula and Mee for all

their friendly and competent help. I would also like to thank the district health teams in Attapeu and Sekong who helped collect so much of the data alongside over a hundred village health workers.

Thanks also to Maude C. Meier at SCJohnson who arranged the donation of both the repellent and placebo lotions used in the trial.

To Sarah Banks (nee Deraedt), a huge thanks for your hard work during your month in Attapeu and for the friendship that's survived wedding planning, new jobs, and even sharing an office. Thanks also to Crystal Lee for keeping me awake during all-night landing catches.

A more personal mention goes to Alex Hiscox, my first friend in Laos and fellow 'mosquito girl' – khop jai der for all the borpenyang chats and lifts on your motorbike. A huge thank you to my parents also for their unstinting encouragement over the past three years. To Andrew goes my eternal gratitude for organising our wedding whilst I was in Laos and for being the best and most supportive husband I could ask for.

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Abbreviations

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
DEET	N,N-Diethyl-m-toluamide (an insect repellent)
ED_{50}	Effective dose required to protect from 50% of mosquitoes
$ED_{99.9}$	Effective dose required to protect from 99.9% of mosquitoes
GMS	Greater Mekong Sub-region (comprising Cambodia, Lao PDR, Myanmar, Thailand, Vietnam and Yunnan in Southern China)
IQR	Interquartile range
ITT	Intention to treat
Lao PDR	Lao People's Democratic Republic
LLIN	Long lasting insecticidal net
LSHTM	London School of Hygiene & Tropical Medicine
OR	Olfactory receptors
PCR	Polymerase chain reaction
RDT	Rapid diagnostic test
SEA	Southeast Asia

UK	United Kingdom
USA	United States of America
VHW	Village health worker
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme

Chapter 1: A literature review examining the suitability of insect repellents for reducing malaria transmission in southern Lao PDR.

1.1 Abstract

Considerable reductions in malaria parasite rates have been recorded in Southeast Asia (SEA) over recent years. However the disease persists in remote, hilly and forested areas which present unique challenges to malaria control efforts. Here mosquito vectors tend to feed outdoors and early in the evening meaning conventional control methods that attack vectors that enter houses, such as long-lasting insecticidal nets (LLINs), do not provide complete protection. Insect repellent used in the evening in combination with LLINs may provide the additional protection required. Five studies of insect repellent, including three randomised controlled trials have previously been carried out in Asia and South America. Significant reductions in malaria rates were recorded in three of these studies meaning the intervention might be effective in the Lao PDR. The highest malaria rates in the Lao PDR are found in the south of the country and the local malaria vectors include *Anopheles dirus* and *An. minimus*. Both species are associated with forest environments and transmission of malaria during transitory forest visits is particularly difficult to control through LLINs alone. Insect repellents are suitable to reduce outdoor biting in the forest and may be an effective control method in southern Lao PDR.

1.2. The global malaria picture

Malaria is a treatable and preventable disease, but remains a serious health burden across the tropics. The World Health Organization (WHO) estimates that approximately 3.3 billion people currently live in areas at risk of malaria transmission, and there were 216 million confirmed cases of malaria in 2010 [1]. The same source reports a decrease of 18% in malaria deaths from

800,000 in 2009 to 655,000 in 2010. However a recent review estimates mortality to be about twice as high, around 1.2 million in 2010, representing a 32% decrease since 2004 [2]. Both sources agree that the burden of disease falls most heavily in Africa where 81% of malaria cases and 91% of malaria deaths occurred.

The Roll Back Malaria (RBM) Partnership was established in 1998 in an attempt to co-ordinate the malaria response on a global scale. Malaria control tools currently the focus of RBM activities include vector control through long-lasting insecticide nets (LLINs) and indoor residual spraying (IRS); chemoprophylaxis for vulnerable groups such as pregnant women; parasitological diagnosis and appropriate treatment with antimalarials [3].

Although there are nine families of antimalarials including twenty-five different drugs still in use, antimalarial resistance is a growing threat to successful treatment and resistance has arisen to all of these drugs. The history of the spread of resistance makes it clear that the absence of effective antimalarials will increase the incidence of both malaria cases and deaths. Resistance to chloroquine, sulfadoxine-pyrimethamine and mefloquine arose within the Greater Mekong Subregion (GMS) before spreading through India and into Africa [4-6]. It has even been suggested that *Plasmodium falciparum* from this South-east Asian region is particularly prone to genetic mutation that could result in drug resistance [7]. Antimalarial resistance results in straightforward increases in disease burden, including increases in hospital admissions, mortality and anaemia [8-11] as well as potentially more frequent malaria outbreaks [12]. There is also an economic cost from loss of working days to the cost of novel drug development [13, 14]. Other more subtle impacts that have been suggested include a loss of confidence in public sector health care leading to an increase in the use of unregulated private healthcare providers who might exacerbate the problem by providing monotherapies or substandard or counterfeit drugs [15].

Artemisinins (belonging to the sesquiterpene lactones chemical family) are currently the most powerful antimalarials available and artemisinin resistance is relatively recent. The first

indication of emerging drug resistance is treatment failure and therapeutic studies in Cambodia and Thailand showed the first indications of a slower clearance of parasites in patients treated with artemisinins [15]. Treatment failure can also be caused by poor patient compliance, insufficient dosages, drug malabsorption or poor quality or counterfeit antimalarials [16]. However, similar results have now been found on the Thai-Myanmar and Myanmar-China borders [15] and the threat of artemisinin resistance is both real and potentially devastating for global malaria control methods.

1.3 Malaria in the Greater Mekong Sub-region

The Greater Mekong Sub-region (GMS) consists of Cambodia, Lao PDR, Myanmar, Thailand, Vietnam and Yunnan Province in Southern China. These countries have much in common as far as their malaria ecology is concerned, but are spread between two World Health Organization (WHO) regions. Therefore the GMS Roll Back Malaria Partnership was formed in 1999 to create a coordinated malaria control strategy across the sub-region [17]. Their main aim was to reduce malaria deaths by 50% from the 1998 levels by 2010 as well as further reducing malaria morbidity and the spread of multidrug resistance. By 2007 malaria deaths had fallen by 60% from 2.2 to 0.8 deaths per 100,000 population alongside a 25% reduction in overall malaria cases [18]. These impressive improvements have partly come about through the massive investments in control programmes initiated by the GMS-RBM Partnership, although background environmental changes such as deforestation, increased urbanisation and increasing economic development will also have had a considerable impact [19].

Malaria in the GMS is strongly associated with environment as the main vectors *Anopheles dirus* Peyton & Harrison, 1979 and *An. minimus* Theobald, 1901 are found in forest and forest fringe areas [19]. Therefore the population at risk are those who live and work in or near to forests including forestry workers, ethnic minorities and political or economic migrants. In Cambodia, Thailand and Vietnam forest work has been found to increase the risk of malaria [20-22]. Deforestation introduces a dynamic component to this system, conversion of land-use

from forest to rice cultivation or rubber plantation could result in a reduction in vector numbers as old habitats are destroyed, or increases in particular vector species favouring the new breeding sites. The process of deforestation also brings a greater number of people into a high-risk area and could lead to an increase in malaria cases [23]. A relatively recent change in land-use in the GMS has been the increase in rubber-plantations, particularly Lao PDR, Myanmar, Thailand and Vietnam [24]. This has lead to increases in vector populations in Malaysia and Thailand and resulting increases in malaria have been recorded in Malaysia [25]. Although *Plasmodium falciparum* is the predominant parasite, *P. vivax* causes a significant proportion of malaria cases meaning parasite based diagnosis is very important for correct treatment [3]. As well as correct diagnosis, current malaria control strategy within the GMS relies on treatment with artemisinin-derived combination therapies, distribution of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) [26].

Malaria control in the GMS faces major challenges including multi-drug resistance, counterfeit or substandard antimalarials, widespread population movement and poor coverage of health care to ethnic minorities [18]. Antimalarial drug resistance is a particular concern in Southeast Asia (SEA) as alleles for resistance to chloroquine spread from SEA to Africa and South America in the 1960s [27] and this migration pattern was repeated with pyrimethamine-resistant malaria [5]. At present the most effective antimalarials are artemisinin-based, resistance to artemisinin has already been detected on the Thai-Cambodian border and is spreading into neighbouring areas [28, 29]. Even more concerning are the first signs of artemisinin resistance genes in *Plasmodium* parasites in Tanzania [30]. If artemisinin-resistance spreads there would be no effective or reliable antimalarials, and for this reason malaria control and elimination where possible in SEA is a high priority. Drug misuse and counterfeiting both contribute to the drug resistance threat in the region. Counterfeit artesunates have been found available commercially in Cambodia, Lao PDR, Myanmar, Thailand and Vietnam [31, 32]. Random sampling in Lao PDR found that 88% of pharmacies sold counterfeit artesunates, although it

should be noted that these collections were carried out in 2003 before the Lao Government had licensed the drug so legitimate sources may have been more difficult to access [33]. However, samples collected in Cambodia showed that fake antimalarials were available in licensed as well as unlicensed shops [34]. Counterfeit artesunates can contain low levels of artesunate or no artesunate at all, as well as a range of other ingredients including paracetamol, other antimalarials and antibiotics [35]. Counterfeit artesunates could endanger the patient taking them, but they can also increase the spread of drug resistance either because they contain low doses of real antimalarials or contain artesunate alone. Irrational drug use is also a problem in the area, monotherapy with artesunate creates a greater risk of resistance as does uncompleted treatment regimes, and these problems have been exacerbated by the increase in private sector penetration in malaria treatment [18]. In addition poor storage conditions could mean even genuine artesunates become degraded and ineffective potentially resulting in under-dosing [36]. Movement of people for both economic and political reasons has resulted in the spread of parasites to new areas and the exposure of non-immunes to infection in highly endemic areas [37-39]. Trans-border movement in particular has been a challenge to national control programmes and particular focus has been given by the WHO to the Thai-Myanmar border and Yunnan borders [18]. The scale of movement is huge, in 2003-2004 there were an estimated 2-3 million migrants within the GMS, a number that is expected to rise with improved highway crossings and visa-free arrangements between countries [40]. Ethnic minorities are also disproportionately affected by malaria in the GMS and unfortunately cultural and language barriers create an additional challenge for health service providers [3, 41]. These populations have often been pushed into hilly, forested areas where contact with *An. dirus* is greater and remote locations make access to health services a serious challenge.

The malaria situation in the GMS remains a serious problem despite recent improvements in morbidity and mortality. The populations at risk are particularly difficult for control programmes to reach and failure of control in this region could have global repercussions.

1.4 Lao PDR

1.4.1 Geography

The Lao PDR shares borders all other GMS countries (Figure 1.1). The Mekong River forms much of the border with Thailand, whilst the Chinese, Vietnamese and Cambodian borders are all hilly forested areas. The Lao PDR has the smallest population within the GMS, only 5.8 million in 2007 [42].

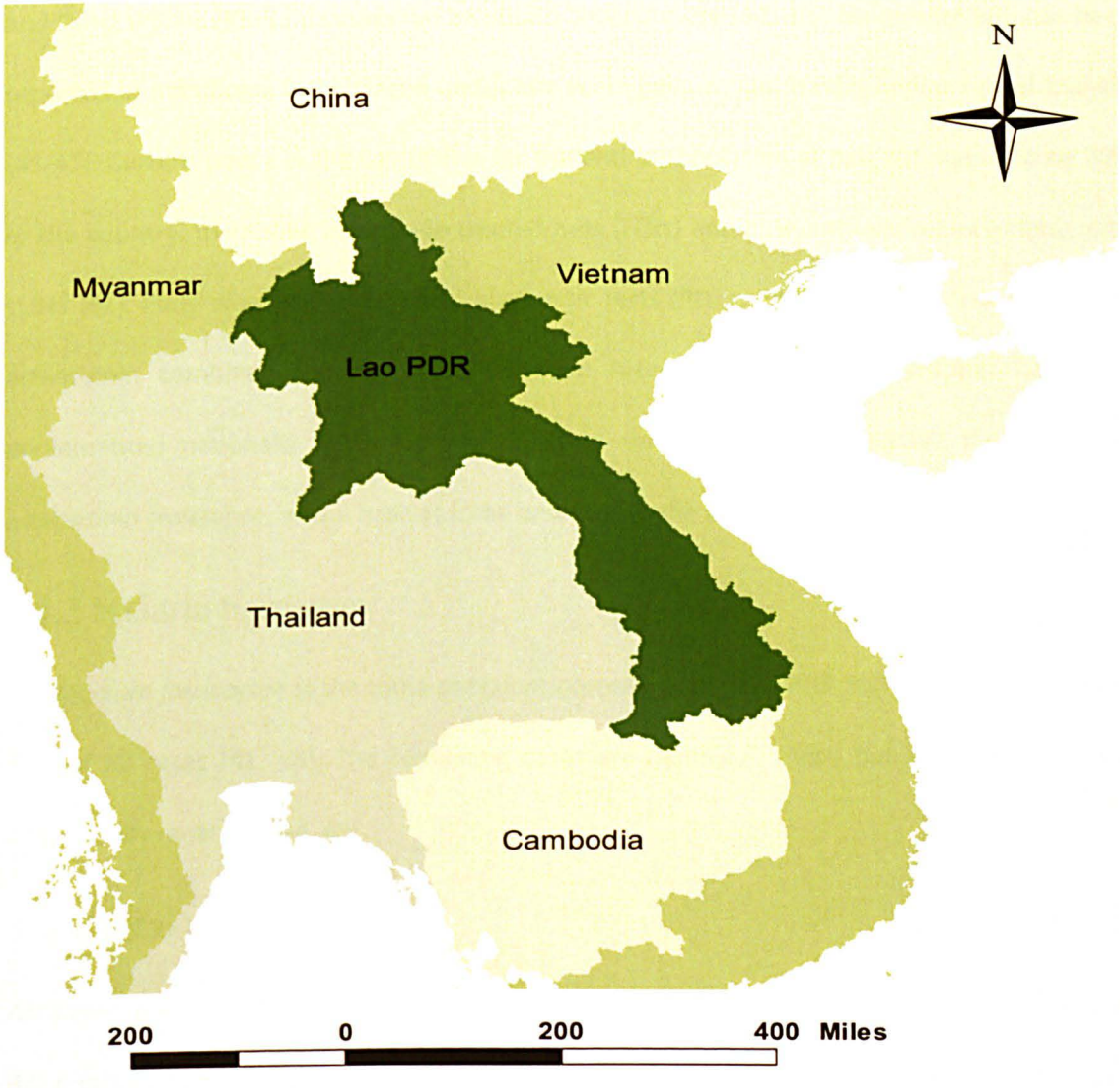


Figure 1.1 The location of the Lao PDR and surrounding GMS countries.

1.4.2 The burden of malaria

In 1998 the Lao PDR had the highest malaria incidence (7.9 cases per 1,000 population) and mortality rate (8.6 deaths per 100,000 population) in the GMS [18]. Although in 2007 the Lao PDR still had the second highest incidence, this represented a substantial improvement as malaria rates had more than halved to 3.3 cases per 1,000 population. Malaria mortality improved even more and the 2007 rate of 0.2 deaths per 100,000 was amongst the lowest in the GMS. Within the Lao PDR the highest malaria rates are found in the two south-easternmost provinces Attapeu and Sekong. In 1998 there were 101 cases per 1,000 in Attapeu and 163 cases per 1,000 in Sekong compared the national average of 55 per 1,000 [43]. Malaria risk factors include non-use of bed nets, sleeping away from home, visiting the forest and living within 2km of a suspected mosquito breeding site [44, 45]. No gender bias has been reported in infections, but children under ten years have a significantly higher risk of malaria [45-47]. Current policy in the Lao PDR is for the entire population at risk, estimated to be 70% of the country, to receive insecticide treated nets (ITNs) and to eventually replace these with LLINs [41]. Early diagnosis using rapid diagnostic tests (RDTs, Paracheck) and treatment with artemisinin combined therapy (ACT, coartem: Artemether and Lumefantrine) has been implemented nationally following pilot trials in three provinces alongside monitoring of artemisinin resistance, which has yet to be detected in the Lao PDR [29, 41].

1.4.3 Malaria parasites

Plasmodium falciparum is the most prevalent parasite in the Lao PDR and is confirmed in over 95% of all cases [41, 48]. The remaining cases are mostly *P. vivax*, but *P. malariae* is also occasionally recorded [44, 49].

1.4.4 Local malaria vectors

Anopheles dirus, *An. jeyporiensis* James, 1902, *An. maculatus* Rattinarithikul & Green, 1987 and *An. minimus* have all been incriminated as malaria vectors in the Lao PDR with *An. dirus* and *An. minimus* likely to be the most important vectors [44, 49, 50]. Members of the *An. dirus*

complex are also major vectors in Thailand, Cambodia and Vietnam [51]. The complex is closely associated with forest environments, traditionally found breeding in forest streams [52] although reports of *An. dirus* larvae in wells may represent a recent colonisation of urban areas [53]. It is thought that dense forest provides dry season breeding sites that allow the *An. dirus* population to persist year round. Feeding times amongst the *An. dirus* complex vary with sibling species, *An. dirus* s.s. and *An. baimaii* bite mostly at night between 21.00h-02.00h but *An. cracens* and *An. scanloni* are early evening biters feeding from 18.00-22.00h [52, 54]. *Anopheles minimus* is also found biting in the early evening, collections from across SEA record peak activity from 18.00h to midnight [50, 55, 56]. Both *An. dirus* and *An. minimus* will readily feed outdoors as well as indoors [50, 52, 56].

1.5. Long-lasting insecticidal nets

There are two methods of manufacture of long-lasting insecticidal nets (LLINs), one where the pyrethroid insecticide is factory applied to the netting material (e.g. PermaNet, Vestergaard Frandsen, deltamethrin coated on polyester) and the second where pyrethroid is impregnated into the fibres before being woven into a net (e.g. Olyset Net, Sumitomo Chemical Co. Ltd., permethrin incorporated into polyethylene) [57, 58]. To be classified as long-lasting the insecticide needs to remain active after twenty washes in the laboratory and after three years standard use in the field [59]. There are two main mechanisms through which LLINs reduce malaria transmission. The first is direct protection of the person sleeping under the net from mosquito biting. Nets treated with pyrethroids provide better protection than untreated nets as the pyrethroid prevents many mosquitoes feeding through the net and reduces entry through holes in damaged nets [60, 61], but this effect is lessened by insecticide resistant vectors and the loss of insecticide through washing or damage to nets [62, 63]. Treated nets can also be manufactured with a larger mesh size allowing greater air passage potentially making them more comfortable to use. The insecticide on the nets will also kill mosquitoes thereby reducing both the size and age of the local mosquito population. This should have the

effect of reducing malaria transmission for all people in the local area regardless of whether they are sleeping under an LLIN or not [64].

Long-lasting insecticidal nets are included in current malaria control strategies in all endemic areas and current policy in the GMS is free distribution to all ages in high-risk populations [48]. Data on LLIN ownership and use are difficult to find and different sources are inconsistent, but the general picture shows there is much more work to be done to reach a good level of coverage of the entire population at risk in the GMS (Table 1.1).

Table 1.1. Current policy towards LLIN distribution in GMS countries and coverage data where available. No information could be found specific to the Yunnan Province of China.

Country	Current bed net distribution policy [1]	Coverage [65, 66]
Cambodia	ITNs/LLINs distributed free of charge to all age groups from 2000	4% of children aged <5 years sleeping under ITNs in 2005. 75% of population sleeping under ITNs/LLINs in high risk areas in 2010
Lao PDR	ITNs/LLINs distributed free of charge to all age groups from 2003	18% of children aged <5 years sleeping under ITNs in 2000
Myanmar	ITNs/LLINs to be distributed free of charge to all age groups (no information on year policy to be adopted)	5.6% of population sleeping under ITNs/LLINs in high risk areas in 2008
Thailand	ITNs/LLINs distributed free of charge to all age groups from 2008	86% of population sleeping under ITNs/LLINs in high risk areas in 2011
Vietnam	ITNs/LLINs distributed free of charge to all age groups from 1992	5% of children aged <5 years sleeping under ITNs in 2006

The evidence base for the disease reduction effect of LLINs is fairly strong, allowing a meta-analysis of malaria health impacts from randomised controlled trials which found that LLINs can reduce clinical cases of malaria by around 50% [67]. Regardless of transmission intensity, LLINs are estimated to save 5.5 lives per 1000 children protected per year. However, there has been discussion about the efficacy of LLINs where vectors are exophilic and zoophilic, as they are in Southeast Asia. Five randomised controlled trials of LLINs in South and Southeast Asia were identified from the literature and their outcomes shown in Figure 1.2 [68-72]. An overall *P. falciparum* reduction of 60% was obtained from a meta-analysis of these trials weighted by sample size (rate ratio 0.41, 95% CI: 0.34-0.49, $p<0.001$). Heterogeneity was low so fixed effects were used to calculate confidence intervals ($I^2=34\%$, $\chi^2=6.10$, $p=0.19$). Thus despite concerns about their efficacy in SEA the evidence shows that they are strongly protective.

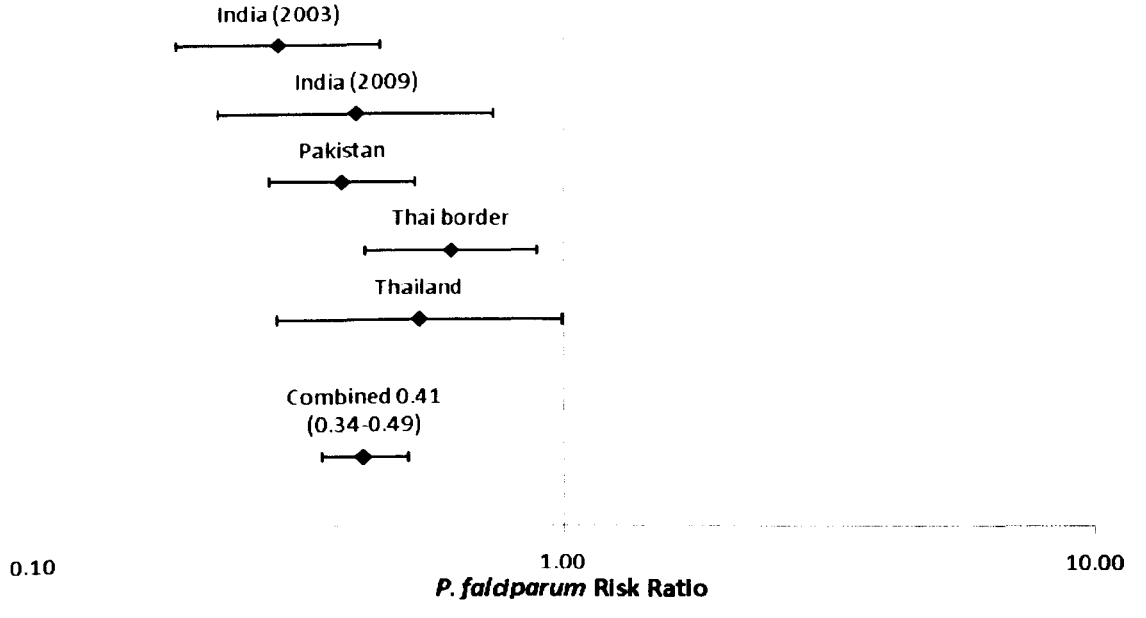


Figure 1.2 The outcomes of randomised controlled trials of LLINs in Southeast Asian countries [68-72]. The combined figure shown is the result of a meta-analysis of the five trials weighted by sample size.

LLINs work best when mosquito vectors bite indoors and at night. Unfortunately in the GMS none of the malaria vectors display these behaviours [56, 73]. When malaria transmission is away from the village, such as in forest habitats, this also shelters vector populations from the community effect of LLINs. So although LLINs are a powerful tool against malaria, in some areas they may not be sufficient as a sole prevention and there is a real need for additional tools.

1.6. Insect Repellents

An insect repellent was defined in 1960 by Dethier as a chemical that "causes insects to make oriented movement away from the chemical source" [74]. For the purposes of this review however insect repellents are defined more narrowly, conforming more to Dethier's definition of a deterrent as a chemical that prevents feeding when placed in a location where feeding would normally take place, and to the common modern use of the term which is a chemical that deters blood-feeding insects from biting.

Repellents are commonly applied to the skin, but can also be burnt to produce repellent smoke deterring insects from a space or applied to clothing or bednets combining the chemical repellent with a physical barrier [75, 76]. Ideally an insect repellent should be non-toxic to humans, non-irritating to the skin if applied topically and non-damaging to fabrics or plastics, whilst at the same time being active against a wide range of insects for several hours [77, 78]. Barnard gives fourteen groups of biting insects and arachnids that should be the focus of repellent research; mosquitoes (Family Culicidae), sandflies (Phlebotominae), blackflies (Simuliidae), biting midges (Ceratopogonidae), horseflies and deerflies (Tabanidae), stableflies (Muscidae), tsetse flies (Glossinidae), reduvid or kissing bugs (Reduviidae), bedbugs (Cimicidae), fleas (Pulicidae), lice (Anoplura), mites (Acarina), hard ticks (Ixodidae) and soft ticks (Argasidae).

Plant-based or natural repellents are mentioned by classical Greek and Roman sources, but are likely to have been used throughout prehistory [79]. The most important natural repellent is pyrethrum derived from *Tanacetum* (or *Chrysanthemum*) *cinerariaefolium* flowers. Pyrethrum is an insecticide as well as a repellent and is commonly used as a quick knock down indoor spray either for personal protection or for insect sampling [76]. As it is quickly broken down in sunlight it has almost no residual effect when used outdoors, so is often used in combination with a synthetic insecticide as its exicto-repellent effect flushes out insects from their hiding places ensuring they receive a lethal dose of the synthetic residual insecticide [76]. Although synthetic pyrethroid insecticides were derived to improve on the short residual life of pyrethrum, modern pyrethroids have a very different chemical structure and molecular mode of action to pyrethrum [80]. Other plant-based insect repellents with proven repellent properties include citronella (oil derived from plants of the *Cymbopogon* genus), lemon eucalyptus (*Eucalyptus maculata citriodon*) and neem (leaves from *Azidarachta indica*) [76]. However, ethnobotanical studies have recorded more than 1200 plants that are traditionally used to reduce insect biting by human populations from all over the world and very few of these have been tested for their repellent or insecticidal properties [76]. One such study in the Lao PDR recorded 91 plant species and one insect species used as insect repellents by rural populations from all over the country, with the most commonly recorded repellent plants being *Nicotiana tabacum* (tobacco), *Sapindus rarak* (soapberry) and *Tadehagi triquetrum* (a legume subshrub commonly used for cattle feed) [81]. Plant-based repellents tend to be cheap and easily available but often do not last long so require frequent reapplication if applied topically, or require a lot of material if burnt as a torch or incense.

Synthetic repellents were first manufactured in the twentieth century, the most successful being DEET (*N,N*-diethyl-*m*-toluamide) developed by the United States Department of Agriculture in the 1950s and still the most widely used repellent today with an estimated 200 million annual applications [77]. There are now a number of synthetic and naturally derived

repellent compounds either already available to consumers or in development, but it is DEET that is used as the gold standard for comparison [82-84]. It is effective against a wide variety of blood-feeding organisms: from mosquitoes and blackflies to ticks, mites and even land leeches [85].

This review is concerned with chemical repellents, but a brief mention of sonic devices is included here for completeness. Electronic devices claiming to repel insects from an area by emitting a high pitched sound first appeared in 1970 and the first experiments showing them to be ineffective were published in 1974 [86]. A Cochrane review including ten studies carried out between 1974 and 2000 found that these devices had no effect on mosquito landing rate in a variety of field locations and conditions [87].

1.6.1 DEET safety and toxicity

DEET has been registered for commercial use for over 50 years, and is used widely across the world [77]. Concerns over the safety of DEET first emerged after reports of encephalopathy following DEET exposure, by the mid-1980s there had been six reported cases of encephalopathy following exposure to DEET all in girls aged 1-8 years which had resulted in three deaths [88]. However the role of DEET in either the illness or deaths was speculative. Ten years later there had been a further eight cases of nervous system toxicity following DEET exposure; no gender bias was found across all fourteen cases, but all except one involved children under 8 years [89]. These cases prompted a number of reviews and investigations of DEET safety.

Of 9,000 calls relating to DEET exposure that were made to American Poison Control Centres from 1985-9, almost 90% were treated solely at home and 80% of those referred to a health centre were discharged after initial examination suggesting mild or short-lived symptoms [90]. The severity of symptoms was found to be more closely related to the type of exposure (inhalation or contact with eyes caused greater symptoms) than the concentration of DEET, or the age or gender of the patient. Laboratory tests have found no reproductive, neurotoxic,

oncogenic or mutagenic effects from DEET use [91]. Animal testing helped establish a no-observed-effect-level for DEET of 200 mg per kg body weight for acute toxicity and 500 mg per kg body weight per day for chronic toxicity, both of which are much higher than estimated average exposures in people [92]. During a clinical trial of DEET for the prevention of malaria in pregnant women, no adverse neurological, gastrointestinal or dermatological effects were reported in the women and neither were there any adverse effects on survival or development of the babies at birth or one year [93]. As part of a re-registration decision The United States Environmental Protection Agency concluded that it was not possible to identify DEET as the cause of the seizures or encephalopathy and that there was no unreasonable risk to human health if used according to product instructions [94]. DEET is now considered very safe for topical use, although it would be prudent to protect younger children from overexposure bearing in mind the seizures that prompted the first safety concerns.

1.6.2 DEET mode of action

Despite its long history and widespread use, the precise mode of action of DEET is not well understood and a number of theories have been tested. Potential modes of action include the inhibition of host signal detection, direct detection activating avoidance behaviour, overloading sensory input so that host signals are lost, or changing host odours to confuse host recognition [95, 96].

Olfactory receptors (OR) are found on the antennae and maxillary palps of mosquitoes [97]. Some are used to detect host odours such as two found in *An. gambiae* Patton, 1905 which detect indole and 1-octen-3-ol, compounds found in human sweat [98]. The electrical response to 1-octen-3-ol OR is reduced when DEET is introduced alongside 1-octen-3-ol [99]. The same experiment with carbon dioxide sensitive olfactory neurones found no change in response. So it would appear that if DEET inhibits host detection, it only works to confuse certain host odours. An odorant receptor has been identified in *Culex quinquefasciatus* Say, 1823 that responds directly to DEET which supports the hypothesis that DEET directly induces avoidance

behaviour [96]. Also in support of this hypothesis, laboratory-reared DEET-insensitive *Stegomyia aegypti* (formerly *Aedes aegypti,* Linneaus, 1762) have been shown to have reduced electrical antennal responses to DEET with no interference with the response of the 1-octen-3-ol olfactory receptor [100]. Antennal response does not allow direct prediction of behaviour response so bioassays are also required to determine whether the detection of DEET actually provokes a particular behaviour. *Stegomyia aegypti* respond to lactic acid, another component of human sweat, as an attractant and have no behavioural response to DEET alone, however a combination of DEET and lactic acid acts as a repellent rather than an attractant [101].

These results suggest that some mosquitoes can directly detect DEET and that it does affect the detection of some host odours. However mosquito species and genera differ in their behavioural response to DEET, so it is possible that the mode of action differs between species. Equally, the mode of actions of other repellents may be different from DEET [95].

1.6.3 Human-mosquito-*Plasmodium* interaction effects on repellency

There is some evidence that DEET may break down faster when used by women compared to men. *Anopheles stephensi* Liston, 1901 were equally attracted to male and female volunteers, but 90% protection from a 32% DEET repellent lasted for significantly longer in men, 9 hours compared to 6 hours in women [102]. In a situation where women were at greater risk of malaria infection or malaria morbidity and mortality, an extremely stable DEET formulation would be desirable. In the GMS and the Lao PDR this is not the case [20-22, 44, 46, 47] so a DEET repellent would be suitable for malaria control, although field test should include both men and women.

Plasmodium-infected mosquitoes are more persistent biters than uninfected mosquitoes [103, 104]. However infection status does not seem to affect response to DEET and the effective dose of DEET required to protect against *P. falciparum*-infected *An. stephensi* was similar to that required for uninfected mosquitoes [105]. The proportion of infective *An. funestus* Giles, 1900 caught in Kenyan field trials was the same from collectors using 5% DEET and ethanol

controls, with a repellency of 49% over 10 hours [106]. Therefore the reduction in biting measured in field experiments is equally applicable to infected and uninfected mosquitoes, and an effective repellent is a suitable method of preventing biting from *Plasmodium* infected mosquitoes and reducing malaria transmission.

The uptake of personal protection methods has been shown to be closely related to biting pressure. In The Gambia at least 30-49 mosquitoes per person per night were required to produce over 80% bed net coverage and the uptake of space repellents was also correlated with mosquito density although only amongst families that did not use bed nets [107]. Mosquito collections in villages in Lao PDR have not reported overall mosquito biting rates only anopheline biting rates, although a study covering sites in eight provinces found 50-80% of collections were culicines [108]. Mosquito biting rates in the forest where malaria transmission occurs are also unavailable. Therefore even if topical repellent is acceptable to local people, close monitoring of patterns of use are desirable to determine whether people chose to use the repellent in high risk areas such as the forest where impact on malaria would be greatest.

1.6.4 Laboratory testing of repellents

The efficacy of a repellent is measured either by the complete protection time (CPT), the time between application of a repellent and first mosquito landing, or by effective dose, the dose required to protect from a percentage of mosquitoes (so ED_{50} is the dose required to protect against 50% of biting and $ED_{99.9}$ is the dose required for almost complete protection). The WHO Pesticide Evaluation Scheme (WHOPES) has provided guidelines for arm-in-cage evaluation of CPT and effective doses [84], however a literature search found no experiments in which these conditions were rigidly followed (Table 1.1). A common deviation from WHOPES recommendations is the definition of treatment failure in CPT experiments. Treated arms are exposed to mosquito cages until a single bite is recorded, but experimenters have regularly used up to four bites to define treatment failure [109-113]. Both effective dose and CPT can be

affected by mosquito species, number of mosquitoes in the cage and cage size. According to WHOPES the number of mosquitoes in a cage can vary from 50-100 to estimate effective doses and 200-250 to estimate CPT. Experiments with *S. aegypti* and *An. quadrimaculatus* Say, 1824 using 50-2,600 mosquitoes per cage found that higher mosquito numbers decreased CPT against *An. quadrimaculatus* but had little effect on protection from *S. aegypti* [109]. A larger cage size decreases protection time against *S. aegypti* biting, but in *An. quadrimaculatus* the longest protection time was recorded in medium sized cages [109]. Mosquito species can also have a huge impact on response to repellents. In a comparison of 18 mosquito species and strains the highest tolerance to DEET was recorded in *An. albimanus* Wiedemann, 1820 (ED_{50}=0.076 mg/cm^2) and differing by almost seven times the lowest tolerance was *Cx. pipiens* Linnaeus, 1758 (ED_{50}=0.011 mg/cm^2) [114]. This comparative low sensitivity of anophelines to DEET compared to other genera is consistent with other studies [115-118].

Figure 1.3 shows the CPT from different concentrations of DEET using laboratory conditions similar to those described above and these tests suggest that a concentration of DEET above 35% would be required to give at least two hours of complete protection against *An. dirus* biting [111, 112]. However the density of mosquitoes is much higher than that found in the field leading to an underestimate of real world protection. Laboratory evaluation of repellents allow standardised comparisons of different repellent compounds and formulation, but field trials are required to estimate protection.

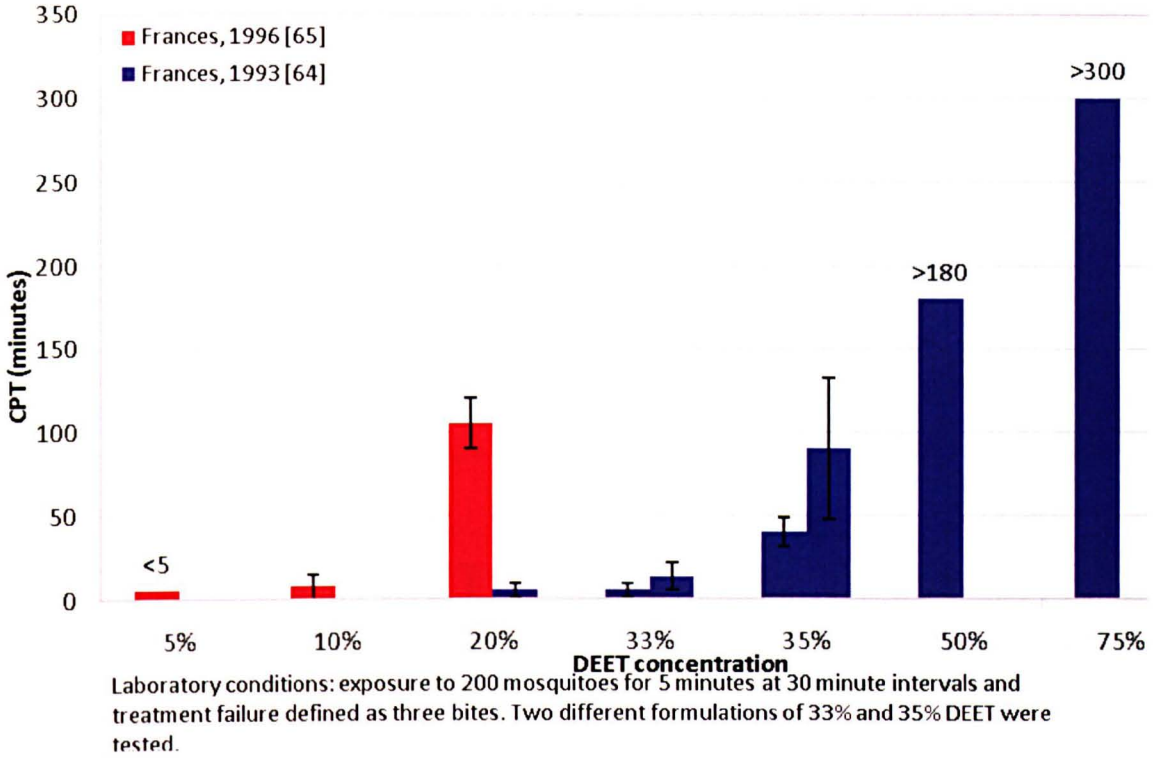


Figure 1.3. Mean ± SE of complete protection time of 5-75% DEET formulations against *An. dirus*.

Table 1.2 Methods used during laboratory testing of repellents compared to WHOPES recommendations.

Study	Exposure time	Rest interval	Treatment failure	Mosquitoes in cage	Mosquito species	Outcome (mean CPT, minutes)
WHOPES guidelines [84]	**3 minutes**	**30-60 minutes**	**1 bite**	**200-250**	**multiple genera**	
Frances, 1993 [111]	5	30	3	200	*An. dirus* *S. albopicta*	5-180+ (20% DEET) 5-240+ (33% DEET) 40-240+ (35% DEET) >180 (50% DEET) >300 (75% DEET)
Frances, 1996 [112]	5	30	3	25-200	*An. dirus*	<5-37.5 (5% DEET) 7.5-172.5 (10% DEET) 105-285 (20% DEET)
Barnard, 1998 [109]	3	90	1	100-1300	*An. quadrimaculatus* *S. aegypti*	270-480 (25% DEET)
Thavara, 2001 [113]	3	30	2	250	*An. dirus* *Cx. quinquefasciatus* *Cx. tritaeniorhynchus* *S. aegypti*	348-870 (20% DEET)
Fradin, 2002 [110]	1	5-15	1	10	*S. aegypti*	88.4 (4.75% DEET) 112.4 (6.65% DEET) 234.4 (20% DEET) 301.5 (23.8% DEET)
Carroll, 2006 [119]	1	30	4	200	*S. aegypti*	120 (10% DEET) 480 (30% DEET)

1.6.5 Field testing of topical repellents

Field trials give a more useful measure of a repellent's efficacy when used in a real situation and usually measure percentage repellency compared to an untreated control using human landing catches [84]. Field trials can also give the duration of a repellent's effectiveness by comparing hourly repellency from application. Although DEET concentration is a factor in the duration of repellency the formulation can have a marked effect as well. A comparison of two commercial products of 34.6% and 40% DEET found that the lower concentration actually performed better over six hours most likely because it was in a more stable formulation [120]. A recurring design problem with field trials is the proximity of treatments and controls. If collectors are too close mosquitoes are diverted from treatment to control artificially distorting the difference between the catches. This diversion effect is recorded in pairs sitting 1m apart but the limit of the effect has not yet been tested [121]. A separation distance of about 15m is recommended based on what is thought to be the limit of short range attraction for host-seeking mosquitoes and WHOPES guidelines say 20m [122, 123]. However as is clear from Table 1.3 most trials have used a smaller distance if they reported it at all. In field trials using a separation of treatment and control over 5m, DEET concentrations of over 15% have been sufficient to produce repellency rates over 85% [124, 125].

Table 1.3. Locations, methods and repellency for field trials in GMS countries including DEET repellents.

DEET concentration	Collection period	Distance from treatment to control	Hours post application	Repellency (%)	Country
7.5%	21.00-01.00h	5m	8	77.6-98.9	Malaysia
	09.00-17.00h	5m	8	46.9-100	[124] *
10%	19.00-04.00h	<1m **	9	100	Thailand
	09.00-17.00h	<1m **	8	90.2-100	[85]
15%	21.00-01.00h	5m	8	91.5-100	Malaysia
	09.00-17.00h	5m	8	85.2-100	[124] *
	18.00-06.00h	'near'	12	85	Vietnam [126]
20%	09.00-17.00h	1m	8	94-100	Thailand
	19.00-24.00h	1m	5	94.2-100	[113]
20% DEET and 0.5% permethrin	18.00-24.00h	10m	6	98.2-99.6	Thailand [125]
25% DEET	18.00-02.00h	Not reported	7	58-93	Thailand [112]
27% DEET	18.00-06.00h	'near'	12	93	Vietnam [126]
33% DEET	18.00-24.00h	Not reported	4-9	87.1-100	Thailand [127]
50% DEET	18.00-24.00h	Not reported	4-9	80.8-100	Thailand [127]
75% DEET	18.00-24.00h	Not reported	4-9	84.6-100	Thailand [127]

*DEET applied to one side of body only and another repellent to the other.

** DEET applied to one side of body and control was other side

1.6.6 Repellents as a malaria intervention

There have been few randomised control trials of insect repellent used to reduce malaria. One of the first used a repellent soap containing 20% DEET and 0.5% permethrin randomised to matched pairs of communities in Ecuador and Peru [128]. Self-reported malaria incidence decreased in both intervention and control communities in Ecuador and increased in both in Peru, with no statistical difference between treatment arms. In Thailand a local cosmetic, thanaka (*Limonia acidissima*), was combined with 20% DEET and used as a mosquito repellent by pregnant women in a Karen refugee camp [129]. The incidence of actively detected *P. falciparum* in women using DEET and thanaka was 28% lower than in those using only thanaka, but this was not statistically significant. The incidence of *P. vivax* was 9% lower in the repellent group, but again this did not reach statistical significance. Repellent soap (20% DEET and 0.5% permethrin) and a placebo lotion were randomised to households in an Afghan refugee camp in Pakistan. This trial found a 58% reduction in *P. falciparum* incidence which did reach statistical significance [130]. No effect was demonstrated for *P. vivax* infections, although this may have been masked by relapsed cases. In a household randomised controlled trial in Bolivia, use of a repellent lotion containing 30% *p*-menthane-3,8-diol (PMD, a repellent compound found in lemon eucalyptus, *Eucalyptus maculata citriodon*) was associated with a statistically significant 80% in *P. vivax* incidence and a non-significant 82% reduction in *P. falciparum* incidence compared to 0.1% clove oil controls [131]. Finally, a case-control study of repellent soap (20% DEET and 0.5% permethrin) in Afghanistan found a significant 92% reduction in the odds of malaria associated with repellent use in the ten days prior to an infection after accounting for use of bed nets and other factors [132].

Low malaria rates in both Thailand and Bolivia mean that although reductions in malaria were recorded these did not reach statistical significance. In Peru and Ecuador there were problems with the formulation of the repellent used, in hot and humid climates it became difficult to use and compliance was even further reduced because only about 50-70% of the required amount

was provided to families each month. These trials show little overall statistically significant effect on malaria rates from repellent use (Fig. 1.3). However non-significant reductions in malaria are explainable by flaws in study design and small sample sizes so the effect of repellent on malaria is not fully explored.

All of these trials used topical repellents, and there have been no published studies of the effectiveness of other forms of repellents such as space repellent or impregnated clothing. The most common use of repellent clothing is by the armed services, so there is a good data to show the effectiveness of these interventions at reducing biting from a range of biting insects [133, 134]. As troops tend to use chemoprophylaxis alongside other interventions, their efficacy at disease prevention in the field is not so well studied and failure often linked to non-compliance [135]. Although the literature on the effectiveness of topical insect repellent is small, there is a huge gap in knowledge of the efficacy of these other repellent delivery systems.

As disease control interventions, repellents have the advantage of being able to protect users outdoors while they are still active. However they have drawback in that the length of protection can be short, requiring reapplication. In addition when used to prevent disease transmission, they can divert biting to non-users thereby increasing disease risk for these people. If no humans are available diverted mosquitoes are likely to feed on animals, and this has been shown even in extremely anthropophilic mosquito species *An. gambiae* s.s. [136]. A mathematical model of repellents used to prevent malaria found compliance to be the most important variable in their success as an intervention [137].

1.7. Discussion

The current malaria control strategy in the Lao PDR relies on free distribution of LLINs alongside free diagnosis and treatment with ACTs [48]. The most important barrier to the use of repellents by people in malaria endemic areas is probably cost [79]. One of the first

attempts to produce a low cost and effective repellent formulation was 20% DEET and 0.5% permethrin soap, which is lathered and left to dry on the skin. Although early trials showed promising results, its efficacy is reduced by physical activity and sweating and many users found the formulation uncomfortable to wear [128, 138, 139]. At present insect repellents are only recommended and widely used by tourists and military personnel visiting endemic countries [140].

Although the numbers of cases and deaths caused by malaria in the GMS seems low when compared with the scale of the disease elsewhere, the threat of antimalarial resistance which would have global implications makes malaria control in this area a very high priority. The southern provinces of the Lao PDR have relatively high malaria rates and are geographically very close to the Thai-Cambodian border where antimalarial resistance has emerged before. Although researchers have struggled to demonstrate an impact on malaria transmission from repellent use, two recent randomised controlled trials have found major reductions in malaria associated with DEET and PMD.

1.8 Study rationale

Southeast Asian malaria vectors are known to bite outdoors and in the evening as well as indoors during the night, meaning LLINs may only provide partial protection from malaria transmission. Repellents used during the evening could provide additional protection over that given by treated bed nets. Following the encouraging results of similar trials in Bolivia and Pakistan, this trial aims to establish whether Southeast Asian malaria vectors can also be prevented from transmitting malaria by the use of insect repellent.

1.9 Aims and objectives

The overall aim of the study is to determine whether insect repellent are a suitable intervention against malaria in southern Lao PDR. The specific objectives are:

1. To establish the most appropriate concentration of DEET for use during evening hours against local vector species in the Lao PDR.
2. To design a household randomised controlled trial to test the effectiveness of repellent lotion to reduce malaria incidence.
3. To compare baseline socio-economic data to ensure randomisation has been carried out fairly.
4. To monitor compliance with repellent use throughout the trial.
5. To monitor adverse reactions to repellent use throughout the trial
6. To investigate acceptance of repellents as an intervention tool by local communities.

1.10 Study Management

The trial design, management, data collection and analysis was the work of a large team of collaborators, so this section aims to make clear the different roles of the people involved. The trial concept was developed by Nigel Hill and Ilona Carneiro at the London School of Hygiene & Tropical Medicine (LSHTM) following a repellent trial in Bolivia. PSI Laos are a social marketing organisation who joined with LSHTM to host the repellent project in the Lao PDR. The study protocol was developed by the Repellent Trial Manager, Vanessa Chen-Hussey (VCH), and approved by PSI Laos and the Lao Ministry of Health. Trial materials such as questionnaires and databases were developed by VCH and translated by Santi Sayarath (SS) the Project Co-ordinator. Prior to the start of data collection, Dr Hongkham Keomanila (DH), a representative from the Lao Ministry of Health's Center for Malariology, Parasitology and Entomology was assigned to liaise with the project. Training of field staff was carried out by VCH, SS and DH. Data collection was largely carried out by district health staff and village health workers who

made monthly visits to perform rapid diagnostic tests, questionnaires and distribute repellent and placebo lotions. VCH, SS and DH visited each of the eight districts once each month to observe village surveys. Support for these visits was commonly supplied by Field Operations Officers from PSI Laos. Evening sniff checks were also carried out by VCH, SS and DH during these field visits. Entomological data collection was carried out by VCH, DH and two MSc students from LSHTM, Crystal Lee and Sarah Deraedt. All data were double entered, with the first set being completed by VCH and SS and the second by an independent data entry company, Viengkham Soomsaath. Data cleaning and analysis was carried out by VCH. Financial management and administrative support were provided throughout the two years of the trial by the finance and administration teams in PSI Laos.

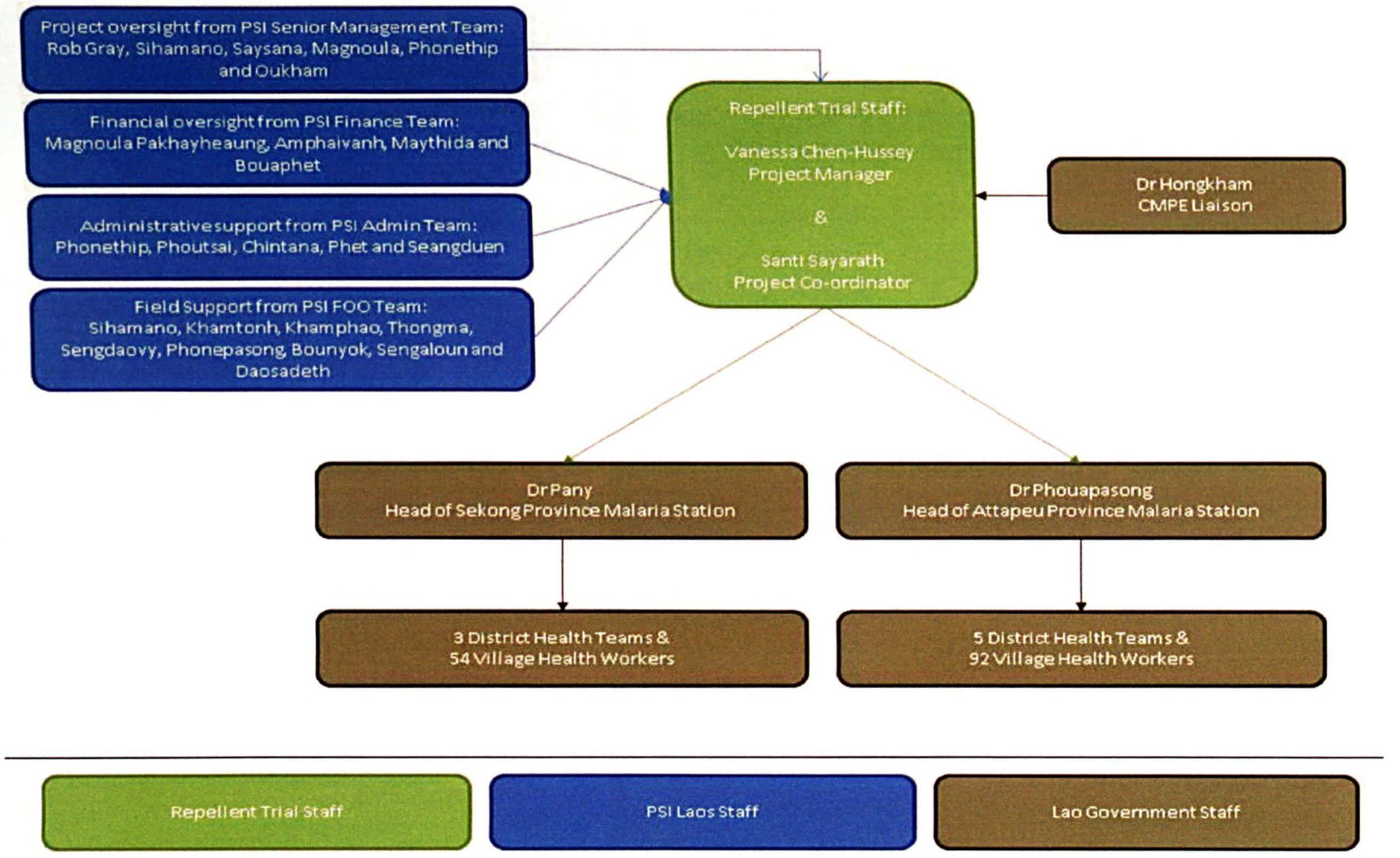


Figure 1.4. Diagram to illustrate staff structure of the trial.

Chapter 2: Entomological surveys to assess the efficacy of *N,N*-diethyl-*m*-toluamide (DEET) to reduce malaria transmission in southern Lao PDR.

2.1 Abstract

The main Southeast Asian malaria vector species bite outdoors and in the early evening before people are protected by long-lasting insecticidal nets. Insect repellent used in the evening could therefore help prevent malaria transmission. In order to support a clinical trial of *N,N*-Diethyl-*m*-toluamide (DEET) used to prevent malaria transmission, we carried out entomological studies in southern Lao PDR to assess the effectiveness of DEET against local mosquito species. Routine entomological surveys using light traps and larval sampling were carried out in July 2009 to gather background information on the species found in the study area. In July 2010 human landing catches with 0%, 10%, 15% and 20% DEET were carried out over sixteen evenings from 17.00h to 22.00h in a Latin-square rotation to compare the efficacy of these concentrations. All DEET concentrations tested gave significant protection from biting compared to the placebo over the five hours of testing. After controlling for night and collector variation, 10% DEET gave 96.1% protection (95% C.I. 92.4-99.0%), 15% DEET provided 98.9% protection (95% C.I. 96.0-100%) and 20% DEET gave 98.1% protection (95% C.I. 95.0-100%). The greater variation found in the protection from 10% DEET means that 15% would be a safer recommendation for use in the repellent trial, where environmental conditions were much more variable and repellent use was mostly unsupervised.

2.2 Introduction

Within the Greater Mekong Subregion (GMS) malaria is largely a rural disease, affecting poor and remote populations; it is highest in border regions which tend to be forest or forest fringe areas with low population densities [18, 141]. In the ten years since the Mekong Roll Back Malaria Initiative was created malaria cases have fallen by a quarter and malaria deaths by almost two thirds [18]. However it is possible that these improvements are more the consequence of deforestation, economic development and improved basic healthcare rather than malaria specific interventions [18]. Control is currently focused on parasite-based

diagnosis of cases, treatment with artemisinin-derived combination therapies, distribution of long-lasting insecticidal nets (LLINs) and indoor residual spraying [3, 18]. The region faces major challenges to malaria control including multidrug resistance, counterfeit antimalarials, widespread population movement and poor health care coverage [18]. The malaria situation in the Lao PDR follows this pattern affecting poor, remote, rural populations, and is highest in southern provinces [141]. The main malaria vectors in southern Laos are *Anopheles dirus*, *An. minimus* and *An. maculatus* [44, 50, 142]. All three species bite outdoors, although *An. maculatus* is the only one to show a strong preference for exophagy [44, 50, 56, 143-149]. The peak biting time of *An. minimus* and *An. maculatus* can start as early as 18.00h [50, 144, 146, 148, 150, 151]; although *An. dirus* feeds later, peaking between 21.00h and 23.00h [44, 49, 50, 54]. Vector feeding outdoors and in the early evening means LLINs would only provide partial protection from malaria transmission. Insect repellent could protect against mosquito biting during the evening hours when people are not yet protected by their bed nets. This is the rationale behind an intervention trial of 15% DEET in southern Lao PDR used to prevent malaria; the repellent is to be used in the evening alongside LLINs at night (Chapter 3: Study protocol).

Although field testing of repellents in GMS countries has been carried out before [85, 112, 124, 125, 127], these tests were necessary to test the DEET formulation used in the trial under local conditions in Lao PDR. In addition most trials have been carried out at night or during the day, with little data from the early evening hours that are particularly relevant to the trial. As the repellent protection in the intervention trial would only be needed for a few hours, lower concentrations of repellent could be more appropriate. Although DEET is considered very safe and has been used by millions of people worldwide for over 50 years [77], concerns over its safety were raised in the 1990s after it was identified as a potential trigger of seizures in a small number of young children [88, 89]. Multiple studies found little risk from normal use (application to skin of commonly applied doses) of DEET [90-94]. However, as no other cause

was definitively identified for the seizures it is desirable to use the lowest effective concentration in an intervention trial involving long term DEET exposure. This study set out to determine whether low doses of DEET are effective against local vectors in the Lao PDR.

2.3 Methods

2.3.1 Study Location

Collections were carried out in and around Gueng Makheua village (14° 44' 32" N, 106° 58' 20" E) and a newer expansion to the village, Makheua Neua, in Attapeu province in southern Lao PDR (Figure 2.1). The village is the central point of four villages, so although its population is only around 900, it has a health clinic and a school. Mosquitoes were also collected from two houses surrounded by rice fields about 3km from Gueng Makheua. These houses allowed village residents to live near their crops throughout the planting and harvest seasons. The number of people in these two houses during surveys varied between two and eleven people. Both rice fields and village were surrounded by open canopy forest.

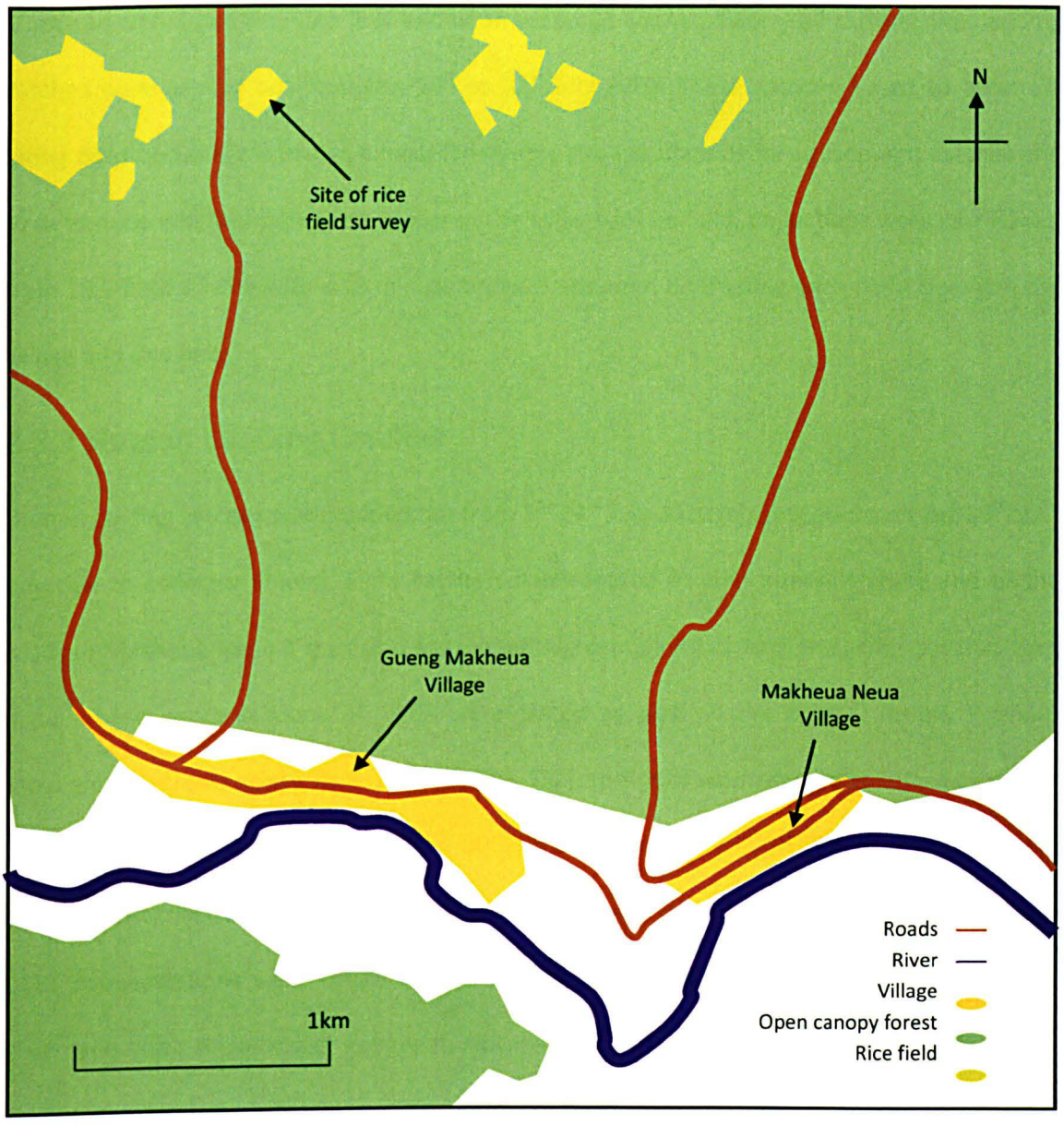


Figure 2.1 Mosquito sampling locations: Gueng Makheua and Makheua Neua villages and rice field site.

2.3.2 Surveys of the local mosquito population

In an attempt to help identify sites with high numbers of vectors, both larval and adult mosquito surveys were carried out. Larval surveys were carried out using larval dippers to sample all aquatic habitats within Gueng Makheua, in the forest surrounding the village and from the rice fields nearby. Ten dips were taken from each habitat identified: roadside ditches, temporary flood pools around the river, undisturbed buffalo wallows and the edges of small streams. CDC light traps were set in randomly selected houses throughout the village from 19.00h to 07.00h from 3rd-29th July 2009 and 7th-21st July 2010. Traps were placed at a height of

1.5m and were 0.5m from the foot end of an occupied bednet. Twelve all night human landing catches were carried out from the 10th to 21st July 2009. These catches aimed to ascertain biting density overnight to give a basis for sample size calculations for subsequent catches and to determine whether biting was higher in the village or rice field. Collections were carried out from 18.00h to 06.00h with a 15 minute break every hour, alternating each night between the village and rice field.

2.3.3 Human Landing Catches

Human landing catches were carried out from 5th-23rd July 2010 (no collections on the 20th-22nd July due to collector illness). Eight catchers were seated at 10m intervals along one of the roads in Makheua Neua (Figure 2.1) for sixteen nights. Collectors kept the same position each night. Three concentrations of DEET were tested as well as the control lotion. Previous repellent trials have used 20% DEET [129, 130, 132], so this concentration was tested alongside two lower concentrations 15% and 10% DEET. The control and 15% DEET concentrations from the repellent trial (formulated and supplied by SCJohnson, USA) were tested. The 10% and 20% DEET concentrations were mixed from a commercially available 50% DEET product (Boots Pharmaceuticals Repel Insect Repellent, Nottingham, UK) and moisturising lotion (E45 lotion, Reckitt Benckiser, Berkshire, UK). Two bottles of each of the four treatments were made up and randomly labelled A-H. The key to the code was kept in a sealed envelope in the field laboratory and not opened until after data analysis was complete. The eight collectors were randomly assigned to one of the eight treatments over the sixteen nights according to two 8x8 Latin squares [152]. Each collector applied 10ml of lotion to their lower legs and arms at 16.45h and wore shorts and shirts that covered them to the knee and elbow. Volunteers collected mosquitoes from their exposed skin from 17.00h to 22.00h with a ten minute break each hour.

2.3.4 Data Analysis

The count data from landing catches were log transformed, and background biting estimated by geometric mean of control catches. A blinded analysis was performed first: the log transformed data were used to build a mixed effects model including date as random factor parameter, and collector (or location) and treatment A-H as fixed effects. Pairwise comparisons with Bonferroni corrections were used to determine whether there was substantial variation in catch size within each pair of treatments. To determine the effect of repellent concentration these analyses were repeated with the DEET concentration as another fixed effect. Percentage protection from biting was calculated from nightly counts and compared using Wald tests on the rate ratios of treatment to controls. Statistical analyses were performed on STATA 11 (Texas, USA).

2.4 Results

2.4.1 Local Mosquito Population

Mosquitoes belonging to 13 genera were captured by light trap and landing catches. *Anopheles, Culex* and *Stegomyia* were identified to species by morphology, and there were 49 species including 25 anopheline species in the catches. Twenty-two of these species are incriminated as disease vectors either in Laos or elsewhere (Table 2.1). *Culex pseudovishnui* Colless, 1957, *Cx. vishnui* Theobald, 1901 and *Cx. whitmorei* (Giles, 1904), all vectors of Japanese Encephalitis (JE), accounted for almost three quarters of all mosquitoes collected. The major malaria vectors *An. dirus*, *An. maculatus* and *An. minimus* were also collected although in very low numbers and made up only 0.3% of mosquitoes biting humans.

Although twice as many mosquitoes were caught by light traps in the rice field houses than in village houses ($z=-17.67$, $p<0.001$) the human landing collections found the opposite with almost three times as many mosquito caught in the village per man hour ($z=2.61$, $p=0.009$). Most mosquitoes were collected from outdoor catches in the village (Table 2.2, $\chi^2=17.5$,

$p<0.001$). Although low catches meant the differences did not reach significance, it was decided that the repellency field trials should be carried out in an outdoor village setting.

Table 2.1 Average catches of key vector species by CDC light trap (n = 301 trap-nights) and human landing collections (n = 784 man-hours). Full data for all species in Appendix C.

Species	CDC	HLC	Disease
Anopheles barbirostris van der Wulp, 1884 / *campestris* Reid, 1962*	0.030	0.001	Malaria
An. culicifacies Giles, 1901	0.007	0.000	Malaria
An. dirus Peyton & Harrison, 1979	0.007	0.000	Malaria **
An. jeyporiensis James, 1902	0.013	0.000	Malaria **
An. kochi Dönitz, 1901	0.146	0.000	Malaria
An. maculatus K / sawadwongporni Rattanarithikul & Green, 1987*	0.053	0.003	Malaria **
An. minimus Theobald, 1901	0.033	0.000	Malaria **
An. subpictus Grassi, 1899	0.010	0.000	Malaria
Armigeres spp.	0.907	0.056	Filariasis
Culex bitaeniorhynchus Giles, 1901	0.010	0.000	JE
Cx. fuscocephala Theobald, 1907	1.728	0.010	JE
Cx. gelidus Theobald, 1901	0.375	0.000	JE
Cx. pseudovishnui Colless, 1957	10.179	0.042	JE
Cx. quinquefasciatus Say, 1823	0.532	0.046	JE, filariasis
Cx. sitiens Wiedemann, 1828	3.601	0.089	JE
Cx. tritaeniorhynchus Giles, 1901	2.203	0.001	JE
Cx. vishnui Theobald, 1901	14.319	0.284	JE
Cx. whitmorei (Giles, 1904)	13.987	0.023	JE
Stegomyia albopicta (Skuse, 1895)	0.037	0.068	Dengue **
All Mosquitoes	50.595	0.749	

* Species not distinguishable by morphology

Disease transmission shown for any SEA country, but ** indicates the species is a vector in the Lao PDR.

Table 2.2 Total number of mosquitoes caught indoors and outdoors in the village and rice field houses. Collections made over 3 nights per location, geometric mean for hourly biting rates and 95% confidence intervals shown in parentheses.

	Village	Rice field
Indoor	51 (GM: 1.89, 1.00-3.55)	32 (GM: 1.19, 0.53-2.63)
Outdoor	88 (GM: 3.78, 2.34-6.12)	12 (GM: 0.67, 0.18-2.45)

2.4.2 Sample size for calculation for field trials

Human landing catches during 2009 caught an average of 3.2 mosquitoes (standard deviation = 1.9) per evening from 18.00h to 22.00h. Four treatments including the control were tested in a Latin square design would require a sample size of 30 man nights per arm to detect a 50% reduction in evening biting at the 95% significance level with 90% power (Table 2.3). To run the experiment over 16 nights would require two collectors per treatment arm per night.

Table 2.3 Sample size calculations for repellent trial, numbers in bold show sample size collected.

Mean no. mosquitoes per evening	Intervention effect	Man nights required
3.2	50%	**30**
3.2	70%	16
3.2	90%	10
3	50%	34
2	50%	76
4	50%	19

2.4.3 Time of biting

All-night catches from the village showed clear peaks in activity in the morning and evening (Figure 2.2). Highest landing rates in were between 18.00-18.45h, the first hour of collection, therefore the repellent field trials were started an hour earlier to ensure good collections of evening biting mosquitoes.

Collections during the repellent assays actually caught most mosquitoes between 21.00-21.50h. This is most likely explained by a difference in the collections of an early evening feeding genus *Armigeres* between the two years; either year on year variations or the change in location within the village could have caused the over 90% reduction in *Armigeres* numbers. There are clear differences between the feeding times of different genera (Figure 2.3), with *Stegomyia* and *Armigeres* feeding early evening and *Culex* starting later. Although Anophelines showed a biting peak mid-evening numbers were actually very low, they made up only 1.4% of all mosquitoes biting humans making any further analysis inappropriate.

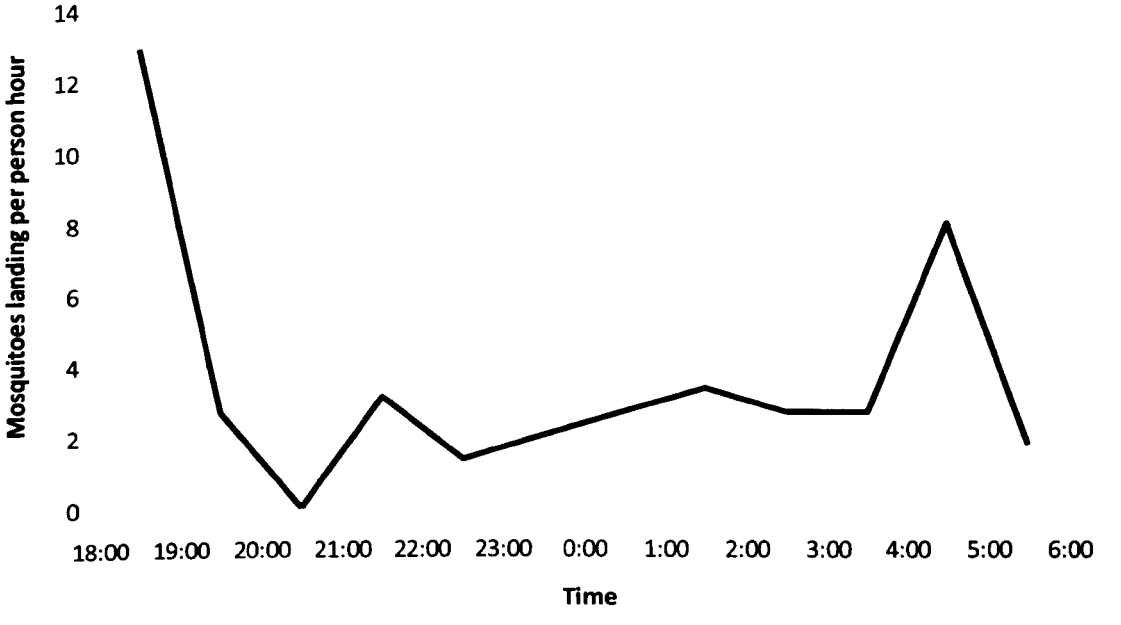


Figure 2.2 All night outdoor human landing catches from the village. Points indicate catches made in previous hour.

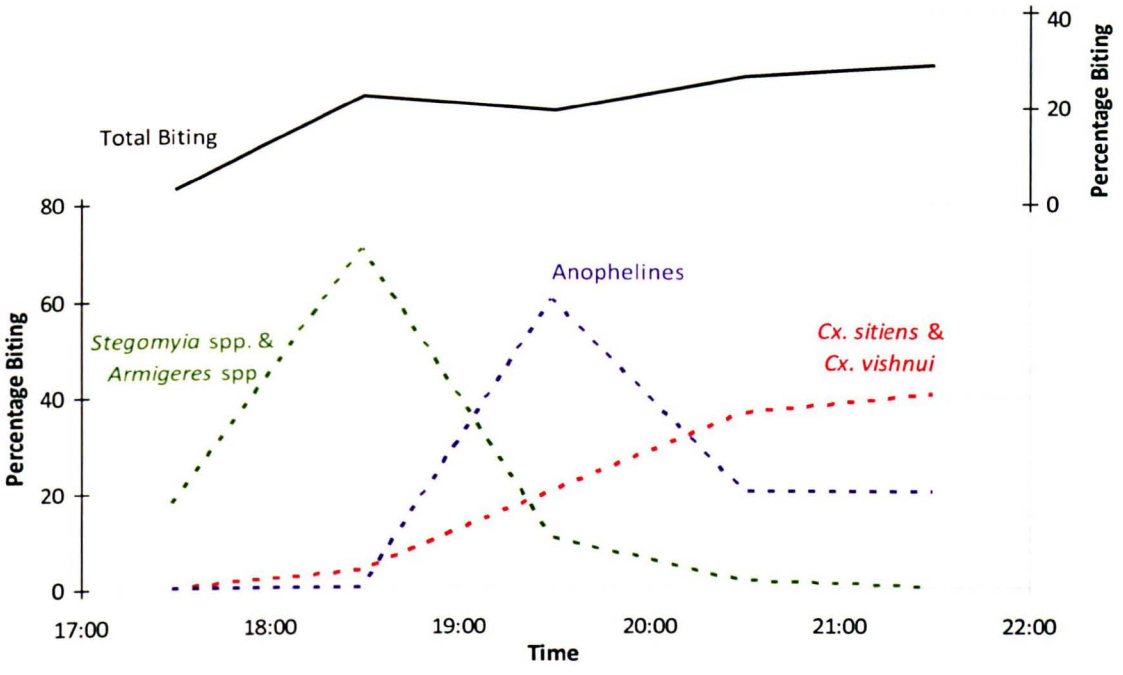


Figure 2.3 Time of biting of different genera 17.00-21.50h. Solid line shows the percentage of the total evening mosquito catch made at each hour. Dotted lines show the same data broken down by genera.

2.4.4 Repellent Field Trial

A total of 10 species were collected biting humans in the evening, 9 are incriminated disease vectors of malaria, JE and filariasis (Table 2.1). Almost half the catch were *Cx. vishnui* (46.8%), other common species included *Stegomyia albopicta* (12.4%), *Cx. sitiens* (12.1%) and *Tripteroides* spp. (11.6%). The repellent effect was high enough on all genera that no significant differences were found, but *Stegomyia* mosquitoes might show a reduced sensitivity to DEET compared to *Tripteroides* or *Culex* species (Table 2.5).

Nightly mosquito catches on volunteers using blinded treatments A-H were significantly different (GLM allowing for variations in night and collector, $p<0.001$, Figure 2.4). The model showed that there was very little variation between collectors. Using collector 8 (who was closest to the geometric mean per night) as a comparison, mosquito catch varied by up to 20%, but the difference was significant in only two collectors. Pairwise comparisons between treatments A-H found that there were no significant differences between the catches using the

same DEET concentrations (Table 2.4). After unblinding, bottles A and E were revealed to contain the control lotion and comparisons between the other treatments found that catches from all DEET concentrations were statistically similar. Controls caught 8.4 mosquitoes per evening (GM, 95% C.I. 6.4-11.0). After adjusting for variation due to night and collector, protection by 10% DEET was 96.1% (95% C.I. 92.4-99.0%, $p<0.001$), by 15% DEET was 98.9% (95% C.I. 96.0-100%, $p<0.001$) and by 20% DEET was 98.1% (95% C.I. 95.0-100%, $p<0.001$).

Lowest repellency was recorded at the first hour of collection for all concentrations of DEET (Figure 2.5) possibly a sampling effect from the low background mosquito numbers, or alternatively because the predominant genera biting at this time, *Stegomyia* and *Armigeres*, do not respond as strongly to DEET. Some reduction was seen in repellency from the 10% and 20% concentrations three hours after application. However protection never fell below 90% for the 15% and 20% formulations.

Table 2.4 Pairwise comparisons of mosquito catches on volunteers using each blinded treatment. DEET concentrations are shown next to the bottle label in parentheses. Comparisons between the same DEET concentrations are shown in red and significant values indicated by *.

	A (control)	B (10%)	C (20%)	D (15%)	E (control)	F (20%)	G (15%)
B (10%)	-1.868*						
C (20%)	-2.117*	-0.249					
D (15%)	-2.124*	-0.256	-0.007				
E (control)	-0.026	1.843*	2.091*	2.099*			
F (20%)	-2.099*	-0.231	0.018	0.025	-2.073*		
G (15%)	-2.210*	-0.343	-0.094	-0.087	-2.185*	-0.112	
H (10%)	-2.067*	-0.199	0.050	0.057	-2.041*	0.032	0.144

Table 2.5 Protection from different mosquito genera by the use of three topical DEET repellents. Total numbers caught on controls are shown as low catches for some genera, mean protection levels appear disproportionately high.

Genera	Total number of mosquitoes caught on controls	% Protection (95% C.I.) 10% DEET	15% DEET	20% DEET
Anopheles Including *An. barbirostris / campestris* *An. maculatus K / sawadwongporni* and *An. philippinensis*	5	100	80 (44.9-100)	100
Armigeres spp.	8	100	87.5 (64.6-100)	100
Culex spp. Including *Cx. fuscocephala, Cx. hutchinsoni, Cx. quinquefasciatus, Cx. sitiens, Cx. vishnui* and *Cx. whitmorei*	252	96 (93.6-98.4)	99.6 (98.8-100)	96.8 (94.7-99.0)
Stegomyia spp. Including *Stegomyia albopicta*	60	88.3 (80.2-96.5)	98.3 (95.1-100)	95.0 (89.5-100)
Tripteroides spp.	45	97.8 (93.5-100)	100	97.8 (93.5-100)
Total	370	95.1 (92.9-97.3)	98.9 (97.9-100)	96.8 (95.0-98.6)

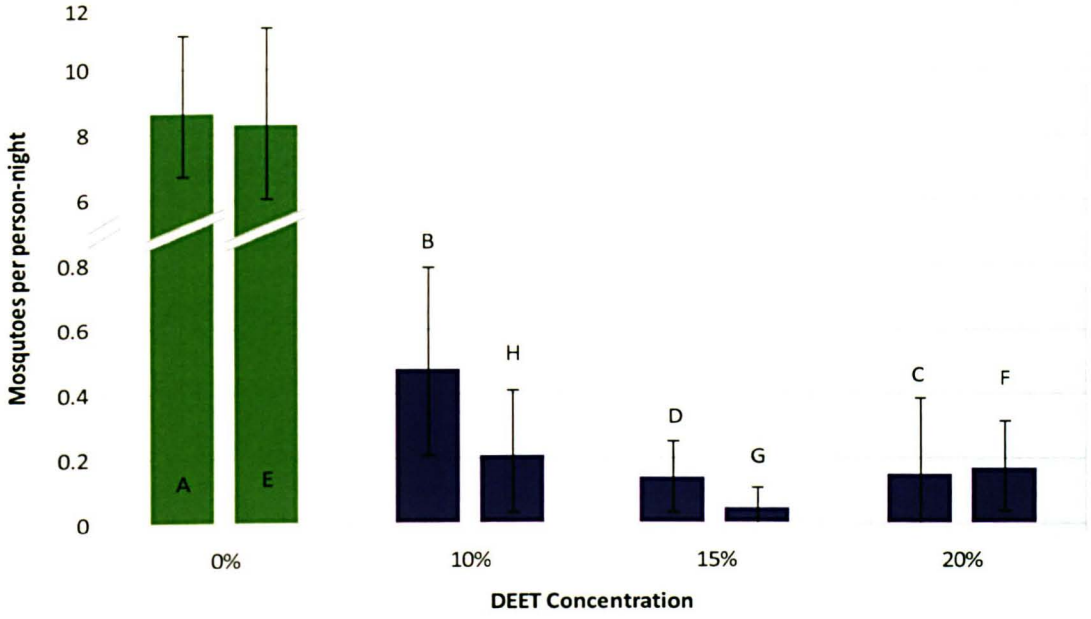


Figure 2.4 Geometric mean and 95% confidence intervals for mosquito catch per person from 17.00h to 22.00h for each treatment.

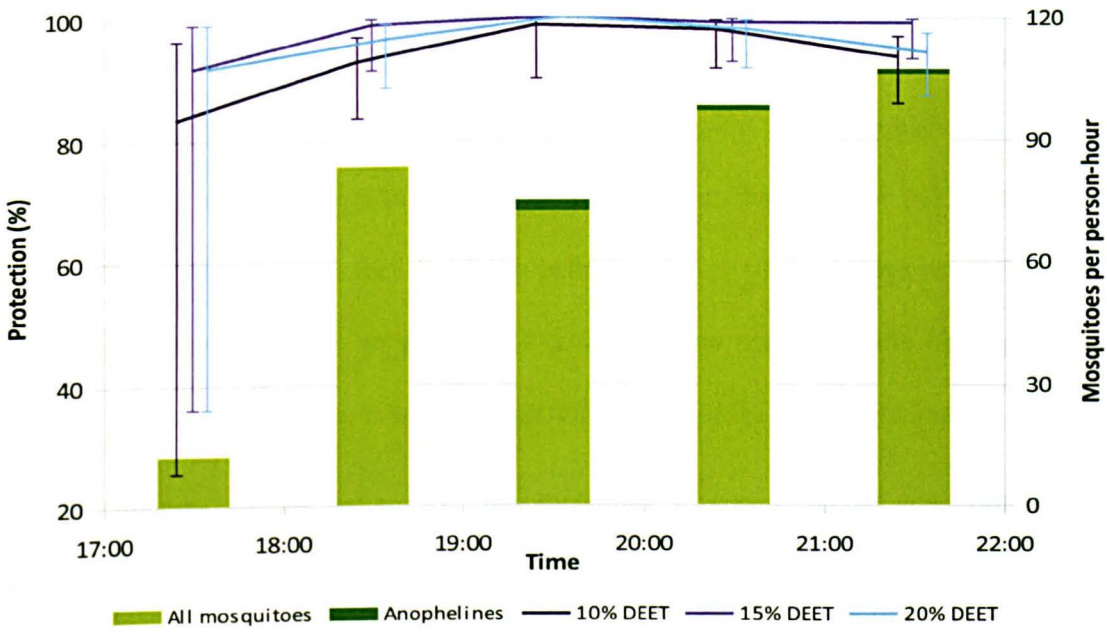


Figure 2.5 Effectiveness of each repellent combination throughout the evening, bars show background level of biting.

2.5 Discussion

Concentrations of DEET from 10-20% provided over 94% protection against mosquitoes biting in the early evening in the Lao PDR. It was not possible to measure protection against malaria vectors as catches of these species were very low. This is consistent with similar trials in Southeast Asia which have found 60-99% protection from 20-33% DEET [112, 125, 127]. The average biting rate on the collectors using the control lotion was 8.4 mosquitoes per person. This is very low when compared with some other field trials in Southeast Asia, where averages of 22-62 mosquitoes per person-night were recorded [85, 124, 127]. Performing repellency trials in areas with high mosquito numbers allows an accurate estimate of protective efficacy to be obtained although it is unlikely to represent the transmission levels experienced in Southeast Asian communities. By performing these tests within a village setting the protective efficacy found in our field trial is likely to reflect the degree of protection that would be obtained in community trials of the repellent.

The repellent trial these tests are linked to is focused on malaria, and unfortunately malaria vectors were very rare in our collections. Anophelines appear to have a higher tolerance to DEET than other mosquitoes [114], so it is concerning that the effect of DEET could not be measured here. However, the fact that the repellent was very successful against general biting is not unimportant. In order to get people to accept and use repellents they have to be perceived to work (Chapter 6), therefore even reducing nuisance biting is an important factor.

Although malaria vectors were rare, the mosquito species collected did contain many important disease vectors in the Lao PDR and other parts of Southeast Asia. Vectors of JE were actually the most abundant mosquitoes feeding on people in the study area: *Cx. vishnui* and *Cx. sitiens* accounted for 49.9% of the human landing collections, compared to malaria vectors which represented only 1.2%. The Japanese Encephalitis Virus is endemic to rural tropical areas of Eastern and Southern Asia, and causes epidemics in temperate areas [153]. It is a growing problem in countries such as the Lao PDR where there is currently no national

vaccination or diagnostic programme [154]. Therefore our results are likely to be relevant to other countries in the region such as Cambodia and Myanmar where JE is also on the increase.

The use of insect repellents as a disease control tool is receiving increased interest, with a number of trials in recent years [130-132]. However, unlike LLINs, where implementation is fairly straightforward, repellent use raises a number of questions: what concentration is best; when and where should the repellent be applied; who should use the repellent; and what form should the repellent take. As the necessity of these collections show, even the concentration of repellent may be difficult to decide on. Our intervention trial took place amongst agricultural workers including whole families including young children and we therefore wanted to identify a lowest effective concentration of DEET. But another important risk group in Southeast Asia are forestry workers [20, 22, 155-157], who tend to be young males and who spend much longer periods of time in the forest. An intervention for this group may therefore involve a much higher repellent concentration in a formulation that is resistant to sweating, perhaps even applied to clothing. This means that repellent will never provide a one-size-fits-all solution that policy makers may be looking for. But this does not mean it has no role to play: our results showed a near 100% reduction in biting, although demonstrating a reduction in disease transmission is a separate and more difficult challenge.

All three DEET concentrations tested gave statistically equivalent protection, reducing biting by over 94% for five hours in the evening. Previous repellent trials have used 20% DEET [129, 130, 132], but our results suggest that 15% or 10% would be just as effective in this rural Southeast Asia setting. Field trials of 10% DEET are very few; only one robust trial including 10% DEET was found by literature search and this was carried out in Australia [158]. Within Southeast Asia the only repellent trial involving a 10% DEET formulation used poor controls and it was therefore not possible to determine a protective effect [85]. In comparison there have been a number of field trials of 15% DEET in Southeast Asia [124, 126] and beyond [159-161] and our results are consistent with their findings. Therefore although 10% DEET performed as well as

20% DEET in these trials, it would be more prudent to use the 15% formulation in an intervention trial.

Chapter 3: Study protocol for a cluster-randomised controlled trial to assess whether the insect repellent *N,N*-diethyl-*m*-toluamide (DEET) can provide additional protection against clinical malaria over current best practice in southern Lao PDR.

3.1 Abstract

Current malaria control efforts in many countries in Southeast Asia, including Lao PDR, focus on long-lasting insecticidal nets (LLINs) and treatment with artemisinin-based combination therapy (ACTs). However, the main malaria vectors in the Lao PDR, *Anopheles dirus* and *An. minimus*, both feed outdoors in the evening before people might be under LLINs and insect repellent could be used to prevent biting during this time. Field trials suggest that 15% *N,N*-diethyl-*m*-toluamide (DEET) should be the lowest concentration effective over five hours. The study took place in Attapeu and Sekong Provinces in southern Lao PDR and was designed to detect whether the use of 15% DEET would result in a 50% reduction in clinical cases of malaria compared with the control group. The study aimed to recruit 800 households in each study arm. Participants were recruited primarily from rural agricultural populations who often work and sleep away from the village during the wet season. Either the 15% DEET or placebo lotion were provided for participants to use every evening. All participants were supplied with LLINs which represents current best practice. Malaria cases were identified through active case detection using rapid diagnostic tests at baseline and at monthly intervals post-intervention. Data collection took place during June to December of 2009 and April to December of 2010. Few previous repellent trials have shown an effect on disease transmission, although in many cases this could be due to problems with trial design, sample sizes and repellent formulations,

but good promising results have come from trials in Bolivia and Pakistan. This trial aims to establish whether repellents could provide similar protection in a Southeast Asian setting.

3.2 Background

Malaria in Southeast Asia primarily affects poor, rural populations [141] and current control efforts focus on LLINs and treatment with ACTs [162]. The main malaria vectors in the region are *An. dirus* and *An. minimus*, which both feed outdoors and in the evening before people might be under their bed nets [142] during which time insect repellent could be used to prevent biting.

Laboratory and field trials of DEET varying in concentration from 5-75% have found that concentrations below 15% do not appear to give useful levels of protection against Southeast Asian mosquitoes [112, 113, 124, 125, 127, 132]. However, different mosquito densities and methodologies make comparisons between the studies difficult.

Few trials have shown an effect on disease transmission from repellent use [128, 129, 132, 159], although in many cases this could be due to problems with trial design, sample sizes and repellent formulations. A South American trial of repellent soap (20% DEET and 0.5% permethrin) found no significant differences between reported malaria cases in intervention and control groups [128]. Only 50-70% uptake of the intervention was estimated during this trial as the soap intervention was found to be unsuitable for active people. Repellent soap of the same formulation was also evaluated in Afghanistan where a 45% reduction in the odds of malaria infection was found in fever patients who used the soap ten days previously; this reduction was not statistically significant, possibly because of small sample size [132]. Similarly, in Thailand, a trial of 20% DEET in thanaka (a local plant-based cosmetic) against thanaka alone recorded a 28% reduction in *Plasmodium falciparum* in the intervention group, but the trial was not sufficiently powered to detect a difference between this and the 15% reduction recorded in the control group [129]. A statistically significant 44% reduction in the odds of *P.*

falciparum infection in households given repellent soap (20% DEET and 0.5% permethrin) compared to a placebo lotion was recorded in Pakistan, although no effect was found for *P. vivax* infection [130]. An 80% reduction in *P. vivax* episodes amongst households given a repellent containing 30% PMD (derived from lemon eucalyptus, *Eucalyptus maculata citriodon*) compared to those given a clove oil control was found in Bolivia, but no significant effect was recorded for *P. falciparum* due to insufficient statistical power [131]. This study was designed to determine whether mosquito repellent applied daily to study subjects in the early evening can protect against malaria vectors that bite outdoors in the early evening before the subjects sleep under LLINs.

3.3 Methods

3.3.1 Study area, house and participant eligibility

Attapeu and Sekong are the most south-eastern provinces in the Lao PDR, sharing borders with Vietnam and Cambodia (Figure 3.9). The Annamite mountains run along the eastern Vietnam border and 60% of Attapeu Province is classed as mountainous [163]. The mountainous areas contain dense rainforest, contrasting with the open canopy dry forest on the plains. River valleys are the most important areas in both provinces for agricultural production, although the regular floods that increase soil fertility can also destroy crops. The wet season runs from April to October, followed by a cool dry season from November to January and a hot dry season from February to March. Rice farming is the main economic activity in these two provinces; 57% of Attapeu's population are farmers and in Sekong the figure is even higher at 71% [43].

Participants were recruited from primarily rural agricultural workers that often work and sleep overnight away from the village during the wet season. Participants were aged 6-60 years old and households had to enrol at least five eligible participants to enter the trial. In addition district health staff were responsible for ensuring that study houses contained at least five

eligible participants, and that study houses were distributed throughout the village, so that study houses were a minimum of 10m apart to prevent diversion of biting from repellent users to non-users [121].

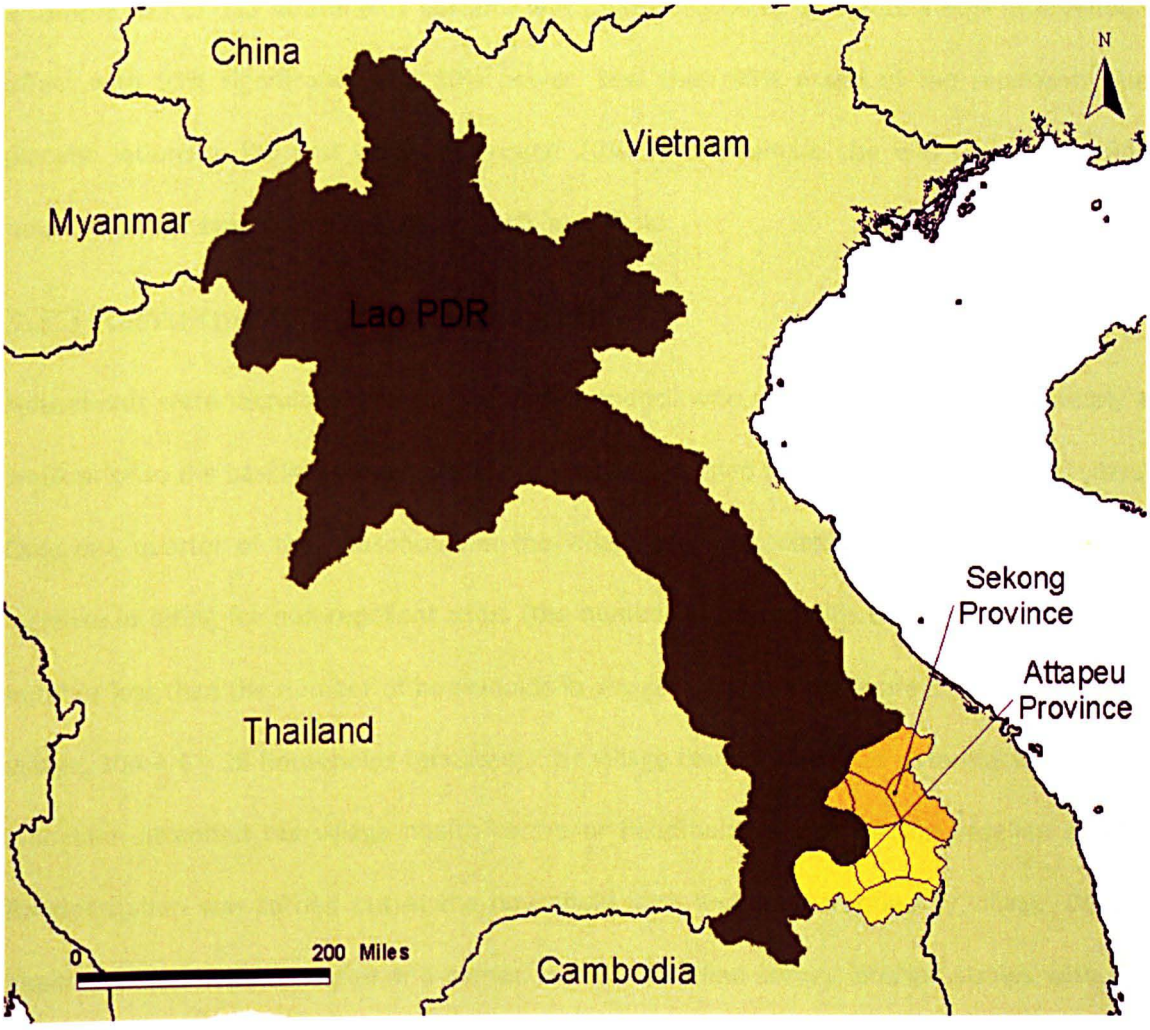


Figure 3.1. The Lao PDR with neighbouring GMS countries. Sekong and Attapeu Provinces indicated.

3.3.2 Sample Size Rationale

The only reliable epidemiological data from the study area prior to the start of the trial was 32% prevalence of *P. falciparum* from village surveys during September 2008 (PSI Laos, unpublished data). Previous trials have shown an 80% reduction in clinical malaria due to *P. vivax* and a 44% reduction in *P. falciparum* infection [130, 131]. It was considered that 30-50% reduction in clinical malaria would represent a useful malaria intervention in this setting. An initial target of 500 households per arm was calculated to be able to detect a 50% reduction in

clinical malaria associated with repellent use at 90% power and 95% significance. This was based on an estimated pre-trial incidence of 2-6%. However, a malaria incidence of only 0.7% was recorded in the first year of data collection. Therefore sample sizes were recalculated and a sample size of 633 households per arm was found necessary to detect a 50% intervention effect with 95% significance and 80% power. Less than 90% usage of the repellents and placebo lotions in the first year was around 20%, so the sample size was adjusted to 800 households per arm, with 5 subjects in each household.

3.3.3 Recruitment and randomisation

Households were recruited through the village council who were consulted approximately a week prior to the baseline survey. Eligible households needed five volunteers aged 6-60 years. Only one quarter of the households in the village were recruited to reduce any potential increase in biting for non-repellent users (the number of households recruited = next whole number less than the number of households in village ÷ 4 e.g. if there are 105 households in village, 104 ÷ 4 = 26 households recruited). The village council identified potential households who then attended the village health centre or headman's house for the baseline survey. Randomisation was carried out at the household level and was stratified by village. District health workers who recruited and carried out the baseline survey labelled straws with the group codes, which the heads of households then picked. Group codes for treatments were "258" or "305" as provided by the manufacturer.

3.3.4 Blinding

The manufacturers held the key that identified the 3-digit code for each lotion, which was revealed after the end of the trial. Trial staff, local health staff carrying out randomisation and surveys, and participants were therefore blinded to the treatment arms. However, the possibility remains that participants were able to distinguish between the active repellent and the placebo, if the effects on biting were clearly different.

3.3.5 Interventions

15% DEET or placebo lotion

A 15% DEET lotion was provided to half the households the other half of households received a placebo lotion (both supplied by SCJohnson, Racine, Wisconsin USA). Although previous repellent trials have used 20% DEET, a 15% DEET formulation was selected because this was the lowest concentration considered effective against Southeast Asian malaria vectors (Chapter 2 and [112, 113, 124, 125, 127, 132]). A low concentration was desirable to minimise the exposure of participants to DEET as the trial would require them to use the repellent for up to nine months. Adult participants were provided with three 100ml bottles of lotion to last one month (approximately 10ml per day). Children under 12 years were provided with two bottles per month, corresponding to approximately 7ml per day. This amount was considered sufficient to apply the treatments to arms and legs as directed by trial staff. District health staff demonstrated the amount of lotion to apply to arms and legs during the recruitment process. Participants were instructed to use the lotion every evening. Full USA compliant consumer product information [94] was given verbally in the local language and printed on the bottle labels in Lao (Appendix B). Any contraindications or side effects were recorded and reported at each monthly follow-up for appropriate action to local District Health departments.

Long-lasting insecticide treated nets

Study households were provided with sufficient LLINs for the household (PermaNet®2.0, deltamethrin 55mg/m^2, mesh 25 holes/cm^2), defined as one net for every 1.5 persons in the household, plus another for use away from home. Participants were instructed to sleep under a net every night, particularly when away from the village.

3.3.6. Design

Three hundred households were recruited from Attapeu province in May 2009, a further 1,297 households were recruited from both Attapeu and Sekong provinces in April 2010. Participants

were recruited as volunteers from meetings with village members and local health team staff where the study was described in full with opportunity for questions. Individuals that wished to take part also received this information in writing and signed a consent form to confirm that they understood the trial, including the possibility that they might receive the placebo lotion. A maximum of 25% of households were recruited from any village to reduce the possibility that mosquitoes are diverted from repellent users to non-users. The baseline survey was carried out at the same time as recruitment. The questionnaire took 30 minutes and collected basic information on wealth indicators and current malaria prevention behaviour for the household, and individual information on malaria exposure. Households were randomly assigned to one of the treatment groups with equal numbers in each arm. Participants were supplied with a month's supply of lotion and sufficient LLINs for the whole household.

After the baseline, Village Health Workers (VHWs) and District Health Staff carried out follow-up surveys at monthly intervals until December in both years, and provided an additional month's supply of lotion. A questionnaire collected data on compliance, acceptability of the lotion and malaria exposure that month. An RDT was carried out on all participants to actively detect malaria cases. Access to remote communities was anticipated to be a major challenge for data collection, particularly during the rainy season. The use of VHWs who are local community members with a few weeks of government medical training, enabled data collection to continue throughout these months. The monthly survey was designed to be as simple as possible to enable VHVs who sometimes have limited education to complete the form. District Health Staff carried out an exit survey during the final month of data collection, to assess the acceptability of the repellent lotion in more detail. VHWs were not used for the exit survey.

3.3.7 Monitoring of repellent and placebo use

The use of the repellent and placebo lotions was monitored by self-reporting in the monthly questionnaires and by estimating the amount of lotion left in the bottles. Lotion use was also directly observed during evening 'sniff checks' carried out by trial staff.

3.3.8. Clinical data collection and patient treatment

Finger-prick blood spots do cause modest, brief discomfort but are common in use in this area and it is likely most individuals have already experienced this. Risk from this procedure is very low and antiseptic wipes were used to prevent infection. The RDTs used were CareStart™ Malaria Pf/Pv Combo test (AccessBio, New Jersey, USA) which detect HRP2 antigen and pLDH and are able to detect both *P. falciparum* and *P. vivax* infections [164]. All positive cases were referred for immediate treatment following local guidelines through the District Health teams working on the study. National guidelines recommend ACT (coartem: artemether and lumefantrine) for the treatment of *P. falciparum* infections in the Lao PDR, and coartem is free at the point of delivery. All positive RDTs and one age- and village- matched negative RDT for each positive were confirmed by polymerase chain reaction at the London School of Hygiene & Tropical Medicine. Use of repellent is common worldwide and presents an extremely low risk to the user. A commercial brand made, registered and sold in the USA by SCJohnson was used in the trial. The lowest effective dose of active 15% DEET was chosen and a consumer friendly gentle aqueous lotion formulation to minimise risk and discomfort. As young children have a lower body volume to skin surface ratio children under six years old were excluded from the trial.

Only the consent forms contained personal information (names) and these were stored for the length of the trial in a locked filing cabinet in the project office in Vientiane. Number codes were used to identify participants during baseline and monthly surveys. Data were entered into a password protected Microsoft Access database.

3.3.9 Analysis

Full details of the data analyses methods are presented separately in Chapter 4. The initial analysis was performed according to intention-to-treat. An additional per protocol analysis was carried out which will include only those participants who slept under a bed net and used the lotion in the evenings at least 90% of the month. Positive cases were subsequently excluded from the trial due to the possibility of them testing positive in later months from the same episode. Comparison of overall malaria, *P. falciparum* and *P. vivax* incidence between the trial arms were carried out by a multi-level mixed effects Poisson regression adjusted for intracluster (household) variation by random effects methods. An assessment of compliance was carried out looking in particular at repellent and placebo use and LLIN use. Adverse events were also be assessed in each arm, separately for children under 12 years and participants over 12 years.

3.4 Ethical approval

Ethical Approval for this study was obtained from the Lao Ministry of Health and the Ethics Committee of the London School of Hygiene & Tropical Medicine (Appendix A). The DEET and placebo lotion were both donated by SCJohnson. The manufacturers did not contribute to the design or analysis of the trial.

3.5 Discussion

Randomised controlled trials minimise bias by keeping participants blinded to treatment allocation and are therefore seen as producing the most rigorous results to inform evidenced based medicine [165]. This trial design is double-blinded meaning that the treatment allocation was concealed from all investigators from data collection in the field to final data analysis. A cluster-randomised design was chosen to minimise potential treatment contamination and interaction. Three bottles of repellent or placebo lotion were provided each month to adults and there is a real risk of mix-up if individuals within the same household were to be given

different treatments. In addition it as been demonstrated that repellents have a diversion effect over short distances and having a mix of treatment within families could artificially increase biting and disease transmission for placebo users.

The recruitment procedures used existing village hierarchy to identify potential households which aided acceptance of the trial at a local level. However, this does introduce potential bias in the households that were recruited. Village councils could preferentially recruit households they perceive as more likely to comply with repellent use; meaning the participants in the trials would be more open to behaviour change than otherwise. As randomisation was stratified by village this issue will not affect results within the trial, but may have some implications for the acceptability of the intervention across the region as a whole.

Monthly RDTs were used to identify malaria cases during the trial. The lack of previous knowledge regarding malaria incidence in the study area made it difficult to judge how frequent testing should be to capture malaria infections in the study population. However, it was hoped that any infections that occurred in between monthly RDTs were picked up by the existing village health services. The same village health volunteers that were seconded for the trial each month also provided RDT testing and treatment as appropriate. Both positive and negative RDT results are entered into village ledgers which can be used to identify malaria cases in the study population that might otherwise be missed. These were used to confirm self-reported malaria in the previous month.

Following the encouraging results of repellent trials in Bolivia and Pakistan [130, 131] this trial aimed to establish whether Southeast Asian malaria vectors could also be prevented from transmitting malaria by the use of insect repellent.

Chapter 4: Analytical plan for a randomised placebo-controlled trial of *N,N*-diethyl-*m*-toluamide (DEET) to reduce malaria in southern Lao PDR.

4.1 Abstract

An initial intention to treat analysis was performed on all data, followed by per protocol analyses which excluded participants whose compliance with long-lasting insecticidal net (LLIN) and evening lotion use was less than 90%, 75% and 50% of the month. A principal component analysis (PCA) using data on education, house construction materials, type of electricity supply, ownership of motorbikes, tractors and televisions and animal ownership was carried out to develop overall socio-economic scores for each household. The PCA scores along with age, gender, nights slept under an LLIN, nights spent away from the village, self-reported lotion compliance and observed volume of lotion used were considered for inclusion in the regression. All variables except treatment group were first tested by non-parametric univariate methods and those with a significant association with the outcome at $p<0.1$ were tested in a multi-level mixed effects Poisson regression model to adjusted for intra-cluster household variation by random effect methods. Variables maintaining their association in the multivariable model at $p<0.05$ were kept in the final model.

Compliance with LLIN use and lotion use were first compared separately between treatment arms and then a chi-squared analysis of the relationship between compliance with each intervention was performed. Focus group discussions suggested that participants used their lotion more when away from the village, so a pairwise-correlation was used to examine the relationship between nights spent away from the village and lotion use. Reports of adverse events were compared between treatment groups using a chi-squared test. A logistic regression was run to examine treatment group, age and gender as predictors for the reports

of allergic reactions. The effect of LLIN use on malaria was estimated by comparison of malaria rates before and after baseline.

4.2 The purpose of an analytical plan

An analytical plan is a detailed description of how the study findings will be analysed. This plan was designed to address the primary objectives of the protocol and was agreed before the start of data analysis. This process reduces the number of different analyses that can be performed on a large data set and hence reduces the probability of a false positive finding. The analytical plan also enables potential design problems or bias to be anticipated as far as possible before the data is collected and analysed, for example with this study the clustering effect of households needed to be taken into account in the analysis method chosen. This process ensures that the results are analysed in an appropriate way and that the results are valid and credible.

4.3 Objectives

4.3.1 Primary Objective

The primary objective of the trial was to detect a 50% reduction in the incidence of *Plasmodium falciparum* infection among 6-60 year olds with 15% DEET repellent compared to a placebo applied topically in the evening, with 80% power at the 5% significance level.

4.3.2 Secondary Objectives

1. To detect a reduction in the incidence of at least one *P. vivax* infection among 6-60 year olds with 15% DEET repellent compared to a placebo applied topically in the evening, with 80% power at the 5% significance level.
2. To compare the incidence of adverse events (allergic reaction, itching or burning) in repellent and placebo users.

3. To compare other negative reports regarding DEET or placebo use such as an unpleasant smell.

4.4 Outline of Study Design

4.4.1 Type of Study

The study was a household randomised, double-blind, placebo-controlled trial of 15% DEET applied topically every evening by rural populations in southern Lao PDR.

4.4.2. Recruitment

Villages with historically high malaria incidence were identified by district health staff using village health centre records (every health centre records RDTs performed and the outcome along with treatment) as potential study villages. Participants were aged from 6-60 years belonging to a household containing at least five eligible members. No more than 25% of the village were enrolled to the trial to reduce the risk of diversion of biting from repellent users to non-users. Trial households also had to be at least 10 metres apart to ensure they were distributed throughout the village. Eligible households were usually identified by the village council.

4.4.3 Intervention

Participants were given three 100ml bottles of either 15% DEET lotion or a placebo lotion (an identical formulation not containing DEET, both provided by SCJohnson) at the start of the trial and the same amount at monthly intervals during follow-up surveys. They were asked at the start of the trial to use the lotion every evening. Trial staff demonstrated how to apply the lotion to arms and legs. Participants were asked to apply the lotion every evening at 18.00h. Each household was also provided with 1 long-lasting insecticidal net (LLIN: PermaNet®2.0, deltamethrin 55mg/m^2, mesh 25 holes/cm^2) for every 1.5 persons in the household plus an extra for use away from the village, and participants were asked to sleep under them every night even when away from the village.

4.4.4 Randomisation

Households were randomised to one of the trial arms by the head of the household choosing straws marked with the intervention codes (258 or 305). Randomisation was stratified by village to ensure that there were equal numbers in each treatment arm, and that village level effects would not influence the outcome of the trial.

4.4.5 Clustering by household

Households were randomly assigned to the repellent or placebo lotions, so all participants within the household received the same treatment. This approach was chosen to minimise the contamination of intervention effects, either through short range diversion of mosquitoes from repellent to placebo users or through confusion of self-administered treatment within the household.

Clustering affects the sample size calculation as statistical power is reduced by the non-independence of individuals. Therefore a cluster inflation factor must be used to produce a sample size. In analysis of cluster randomised trials, failure to control for the cluster effect can lead to a high Type I error rate, leading to rejection of a true null hypothesis.

4.4.6 Measurement of outcomes

All participants were asked to attend a monitoring survey at a local health centre were they are tested for the presence of malaria using a rapid diagnostic test (RDT: CareStart™ Malaria Pf/Pv Combo). The outcomes of interest are the incidence of *P. falciparum* malaria infection, and the incidence of one or more *P. vivax* infections per person-month of observation. Adverse events from repellent use was measured as reported allergic reactions per person month. Allergic reactions were defined as a rash or any sensation of itching or burning on the skin during or after using the lotion. Other reported complaints (bad smell, no effect on biting) were also measured per person month.

4.4.7 Sample size rationale and calculation

During a five month follow-up period in 2009 the incidence of *P. falciparum* infections was 11/6950 = 0.0016 episodes per person-month, and 9/6950 = 0.0014 for *P. vivax*. There was one mixed infection giving an overall incidence of both species of malaria of 0.0029 cases per person month. A similar incidence in 2010 would give 0.024 cases per person over the eight months of follow-up, so a range of expected incidences from 0.015-0.025 cases per person was used in the calculation.

In Pakistan a 44% reduction in the odds of a *P. falciparum* infection was found in households using a repellent soap (20% DEET and 0.5% permethrin) compared to those using a placebo lotion [130]. No effect was found on the odds of *P. vivax* infections in this trial. However, an 80% reduction in the incidence of *P. vivax* was found in a trial of PMD (a repellent derived from lemon eucalyptus, *Eucalyptus maculata citriodon*) in Bolivia [131]. A conservative target of 50% reduction in malaria has been used here as the lowest reduction that would be considered useful as a public health intervention.

The coefficient of variation (k) of the rate of cases between clusters (households) is measured as the standard deviation divided by the mean of each treatment group and is assumed to be similar in the two groups. A k of 0.25 suggests that true cluster rates are normally distributed, so that 95% of rates lie within two standard deviations of the mean [166]. A higher k would mean rates varied more, and a lower k would indicate less variation. In the 2009 data, malaria cases showed clustering at the village but not household level, suggesting that cases are not over-dispersed by household. Therefore the coefficient of variation (k) was set at 0.25.

Non-compliance in the first year of the study was approximately 20% and samples were adjusted to take this into account. A sample size of 790 households per arm will be required to show a 50% intervention effect at 5% significance and 80% power [167]

Table 4.1 Sample size calculations for different scenarios of baseline incidence and intervention effect (with 80% power, 5% significance and co-efficient of variation = 0.25).

Assumed incidence	Intervention effect	Households per arm	Adjusted for 20% loss
0.010	50%	948	1185
0.015	50%	633	791
0.020	50%	476	595
0.010	40%	1579	1974
0.015	40%	1054	1318
0.020	40%	792	990
0.010	30%	2982	3728
0.015	30%	1991	2489
0.020	30%	1495	1869

4.5. Analysis of Baseline Data

A principal component analysis (PCA) was used to develop an overall socio-economic score for each household [168]. Eighteen variables from the baseline survey were entered into the PCA (Table 4.2). Only surveys with complete responses can be entered into a PCA, therefore to avoid systematic bias any variables with more than 20% missing data were not be used in the analysis.

Table 4.2 Variables from the baseline surveys that will be considered for entry into a PCA.

Subject Area	Items from Baseline Survey
Household Size	Household size
Head of Household	Occupation and education
Housing	Roof and wall material
	Electricity and water supply
	Cooking fuel
Possessions	Motorcycle, tractor, radio and television ownership
	Insecticide treated bed net ownership
Animals	Buffalo, pig, goat, chicken and dog ownership

Remaining baseline information on household-level (use of personal protection not including repellents or LLINs) and individual-level characteristics (age, gender, visiting the fields or forest, and RDT results) were compared between treatment arms using chi-squared tests for categorical variables and t-tests for continuous variables. The socio-economic scores from the PCA were compared by non-parametric test.

4.6. Analysis of incidence data

4.6.1 Data Exclusions

Data were excluded from the analysis if the participants withdrew consent and discontinued with the trial. Concurrent infections in the same individual were also excluded in order to avoid counting recurring infections as new cases.

4.6.2 Case Definition

A *P. falciparum* infection was defined as a *P. falciparum* positive RDT detected at a monthly monitoring survey. A *P. vivax* infection was defined as a *P. vivax* positive RDT detected at a monthly monitoring survey. An adverse event was a report of an allergy from lotion use during a monthly monitoring survey. Other adverse reactions to the lotion that were reported at monthly monitoring surveys were; disliking the smell; or it not stopping insect bites; this was an open survey question so other reports may also be given.

4.6.3 Person-time at risk

Once a *P. falciparum* infection was detected, the subsequent month of observation for that participant was excluded from the analysis to avoid double-counting the same infection. So as to avoid recording a relapse of *P. vivax* as a new infection, the analysis looked only at the incidence of at least one episode of *P. vivax* during the trial, but all qualifying months of observation were included in the denominator. If a participant misses a monitoring survey or

does not have a supply of repellent for a particular month, then that month will be excluded from the analysis.

4.6.4 Inclusion of observations

The main analysis was an intention to treat (ITT) analysis, including all qualifying months of observation (see above). The secondary analysis was according to protocol (ATP) based on 90% compliance with application of the repellent or placebo as reported by the participants each month. For this analysis, a month of observation where the lotion was applied less than 90% of the time (e.g. <27/30 days) will be excluded from both the numerator (contributing cases) and the denominator (contributing person-time at risk). Subsidiary analyses were undertaken to look at the effect of the intervention with 75% and 50% compliance with product application per month.

4.6.5 Regression analysis with random effect model

The statistical test used to investigate the effect of the intervention was a multi-level mixed effects Poisson regression model adjusted for intra-cluster variation by random effect methods. This model allows for repeated measures over time on each individual. Household clustering can be controlled for using random effects. The results of the model were presented as the incidence rate ratio of the outcome being considered in the intervention group compared with the placebo group. The co-variables to be considered for inclusion in the model were age, gender, socio-economic PCA scores, percentage of nights that a participant slept with a LLIN, percentage of nights slept away from the village at rice fields or the forest, self-reported lotion use and observed volume of lotion used. All variables were tested in univariate models, and those with a significant association with the outcome at $p<0.1$, tested for inclusion in the final model. Variables maintaining their association in the multivariable model at $p<0.05$ will be kept in the final model.

4.7. Analysis of co-variables

4.7.1 Compliance with LLIN use

The proportion of self-reported fully compliant participants was compared between the two treatment arms using a chi-squared test. LLIN use was converted to proportion use per month to aid comparison with lotion use and considered for inclusion as a co-variable in the regression model.

4.7.2 Compliance with lotion use

Compliance with daily lotion use was not expected to be as high as with bednets because there is not the same history of use in the study area. Compliance was first quantified as the number of evenings lotion was used per person per month. As lotion use might be affected by seasonal changes like temperature or mosquito density, the analysis was also adjusted for month. Focus groups discussions have also suggested that participants used the lotion more often when working in the forest or away from their village. Therefore, a multiple regression analysis was carried out to compare the mean number of evenings when lotion was used between treatment groups, adjusted for month and travel away from the village.

4.8. Analysis of adverse events

4.8.1 Allergic reactions to repellent and placebo lotions

The analysis aimed to determine whether treatment group, age or gender had any effect on the reporting of allergic reactions. Allergies were expressed as present or absent for each participant each month and a logistic regression using random effects run to detect any association with the above variables.

4.8.2 Other negative reports from lotion use

Any other negative effects from lotion use reported by participants - such as headaches, tingling sensations or an unpleasant smell - were recorded for each participant-month. They will also be compared across intervention groups, age and gender.

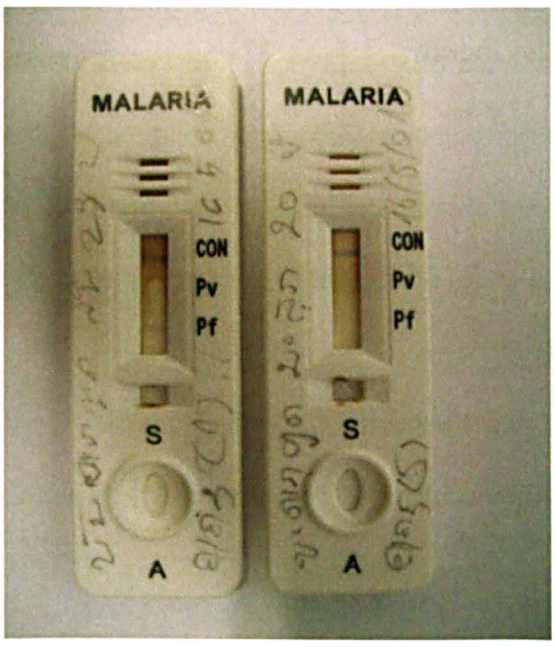
4.9 Unblinding of the Trial

The code for the repellent and placebo lotions was broken after all data had been cleaned and a 'frozen' blind data set sent to an independent statistician as an official record.

4.10. Conclusions

The Poisson regression was chosen because it models rare events, as the malaria rates were expected to be given the incidence in year one. The multi-level, mixed effects model allows for both repeated measures over time and household level clustering to be taken into account. Variables considered for inclusion in the model were informed by previous studies which identified age, net use and forest visits as risk factors for malaria [44, 46, 47, 169]. Although in the Lao PDR studies have found that malaria risk is similar between men and women, in other countries in the region men are significantly more likely to suffer malaria infection and this variable was therefore considered important enough to include in this analysis. The analytical plan provides an overall framework to approach the analysis ensuring fair and rigorous treatment of the data.

Chapter 5: A cluster-randomised placebo-controlled trial to assess whether the insect repellent *N,N*-diethyl-*m*-toluamide (DEET) can provide additional protection against clinical malaria over current best practice in southern Lao PDR.



5.1 Abstract

The main vectors of malaria in the Greater Mekong Subregion (GMS), *An. dirus* and *An. minimus*, bite outdoors in the early evening before people are under bed nets. Long-lasting insecticidal nets (LLINs) are the first choice malaria prevention tool, and vector behaviour such as this enables mosquitoes to avoid contact with nets is therefore a concern. Insect repellent is a potential tool that could be paired with LLINs in areas of outdoor and evening biting. A double blind, household randomised, placebo controlled trial of insect repellent to reduce malaria was carried out in southern Lao PDR to determine whether the use of repellent and long-lasting insecticidal nets (LLINs) could reduce malaria more than LLINs alone. Three hundred households were recruited in June 2009 and a further 1,297 in April 2010 giving a total of 1,597 households which included 7,979 participants. In total 795 households (3,972 participants) were assigned to use a 15% DEET lotion and 802 households (4,007 participants) a placebo lotion. Randomisation was stratified by village and participants. Participants, field staff and data entry clerks were all blinded to the group assignment. All households received new LLINs the current best practice for malaria control in Lao PDR. Participants were asked to apply their lotion to exposed skin every evening from 18.00h. Adults were provided with 300ml per month and children under 12 years with 200ml of 15% DEET or the placebo. *Plasmodium falciparum* and *P. vivax* cases were actively identified by monthly rapid diagnostic tests. Five rounds of follow-up were completed in 2009 and eight in 2010. Intention to treat (ITT) analysis included 1,398 households and no effect from the use of repellent was found on malaria incidence (IRR: 0.97, 95% CI: 0.55-1.73, p=0.926). A higher general socio-economic score derived from principle components analysis (PCA) was significantly associated with a decreased risk of household malaria (p=0.005). According to protocol (ATP) analysis which excluded participants using the lotions less than 90%, 75% and 50% of the time included 1,311, 1,342 and 1,368 households. No effect from the use of repellent was found, while lower socio-economic score increased malaria risk. Therefore repellents are not a suitable intervention in

addition to LLINs against malaria in agricultural populations in southern Lao PDR, and these results are likely to be applicable to much of the GMS. That topical repellents are very effective at reducing mosquito biting is not in question, but this trial provides no evidence that they are an effective intervention against malaria in Southern Lao PDR. Malaria rates were sufficiently high given the required sample size to detect an intervention effect of up to 50%. Potential reasons for the lack of effect include non-standard self-application of repellent that could result in under-treatment or non-compliance with the intervention.

5.2 Introduction

Malaria continues to be a major public health problem in the GMS which unites southern China, Cambodia, Lao PDR, Myanmar, Thailand and Vietnam [18]. These countries have common environmental conditions which create a similar malaria epidemiology across the region. Malaria in the GMS is highest in remote border areas where forest and low population densities create particular challenges to control efforts [21, 51, 157, 170]. The GMS Roll Back Malaria Partnership was set up in 1999 to reform control programmes across the region, including improving surveillance, increasing long-lasting insecticidal net (LLIN) coverage and targeting of risk groups [26]. Between 1998 and 2007 cases of malaria dropped by 25% and malaria mortality by 60% across the region [18]. Whilst effective malaria control has undoubtedly contributed to this reduction, it is suggested that the reductions in malaria may have been helped by other changes including deforestation, economic development and improved basic healthcare [19]. At present malaria control is focused on parasite-based diagnosis of cases, treatment with artemisinin-derived combination therapies, distribution of long-lasting insecticidal nets (LLINs) and indoor residual spraying [3, 26].

The Lao PDR shares borders with all other GMS countries (Figure 5.1) and the highest malaria incidence rates are found in Attapeu and Sekong provinces along the southern borders with Cambodia and Vietnam [18]. *Plasmodium falciparum* causes almost 97% of cases and *P. vivax* the remainder [162]. Village based surveys in Attapeu have found increased malaria risk to be

associated with sleeping without a bed net and visits to the forest [44, 45]. Unusually for the GMS where young men are most at risk of malaria infection, there is not a gender bias in the Lao PDR where young children are the group with the highest rates of malaria [44, 45, 47]. The main vector is *Anopheles dirus* which is strongly associated with forests and is frequently found biting outdoors [52, 142]. Biting time varies depending on sibling species, whilst for most peak biting occurs from 21.00-02.00h, other species start feeding at 18.00h [52, 54]. *Anopheles minimus* and *An. maculatus* are also important vectors and are similarly found biting outdoors in the early evening [44, 50, 56, 108, 171]. LLINs protect from biting indoors at night and also reduce the local mosquito population, protecting even those in the community not using nets [172]. However in the GMS the efficacy of LLINs may be reduced as vector contact is outdoors and the community effect may also be diluted by forest vectors found away from villages [173].

Insect repellents have the potential to reduce vector contact in this setting. Field trials in Thailand and Malaysia show DEET concentrations of 15-20% decrease biting by over 83% [124, 125, 138]. However few trials have been able to demonstrate an effect on malaria transmission by the use of insect repellent. On the Thai-Myanmar border, pregnant women were given either thanaka (a traditional cosmetic derived from *Limonia acidissima*) mixed with DEET or thanaka alone. Although a 29% reduction in *P. falciparum* was observed, the transmission level was too low for this to be statistically significant [129]. Similarly, in Afghanistan low malaria rates meant a 45% reduction in malaria (96% of cases were *P. vivax*) observed in people using a repellent soap containing 20% DEET and 5% permethrin was non-significant [132]. In Ecuador and Peru a village randomised trial of repellent soap found no reduction in malaria compared to untreated controls [128]. A 56% reduction in the odds of *P. falciparum* infection was found in Pakistan amongst households using repellent soap compared to those using a placebo, no effect was found for *P. vivax* infections [130]. Households using 30% PMD (a repellent derived from lemon eucalyptus, *Eucalyptus maculata*

citriodon) in Bolivia had an 80% lower incidence of *P. vivax* [131]. There was also an 82% reduction in *P. falciparum* but case numbers were too low to reach significance. A number of common problems have affected the results of these trials. Lower than expected malaria rates have resulted in insufficient sample sizes and non-significant reductions. Compliance is also very important, since repellent requires application every few hours it is easy to forget, lose and even apply in insufficient doses. The inconsistency of these results means that it is not yet established whether the use of insect repellent can reduce malaria infection. The aim of this trial was to determine whether using a 15% DEET repellent, established by landing catches to reduce mosquito biting by 98.9% (Chapter 2) would reduce malaria incidence against exophagic vectors amongst rural populations in southern Lao PDR using LLINs.

5.3 Methods

5.3.1 Trial Design

Summary

This was a double blind, household randomised, placebo controlled trial of insect repellent to reduce malaria, carried out in southern Lao PDR. Half the households were assigned to the repellent group and the other half to the placebo lotion (Figure 5.1). Reporting of methods, analysis and results has been according to CONSORT guidelines for the reporting of randomised controlled trials [174]

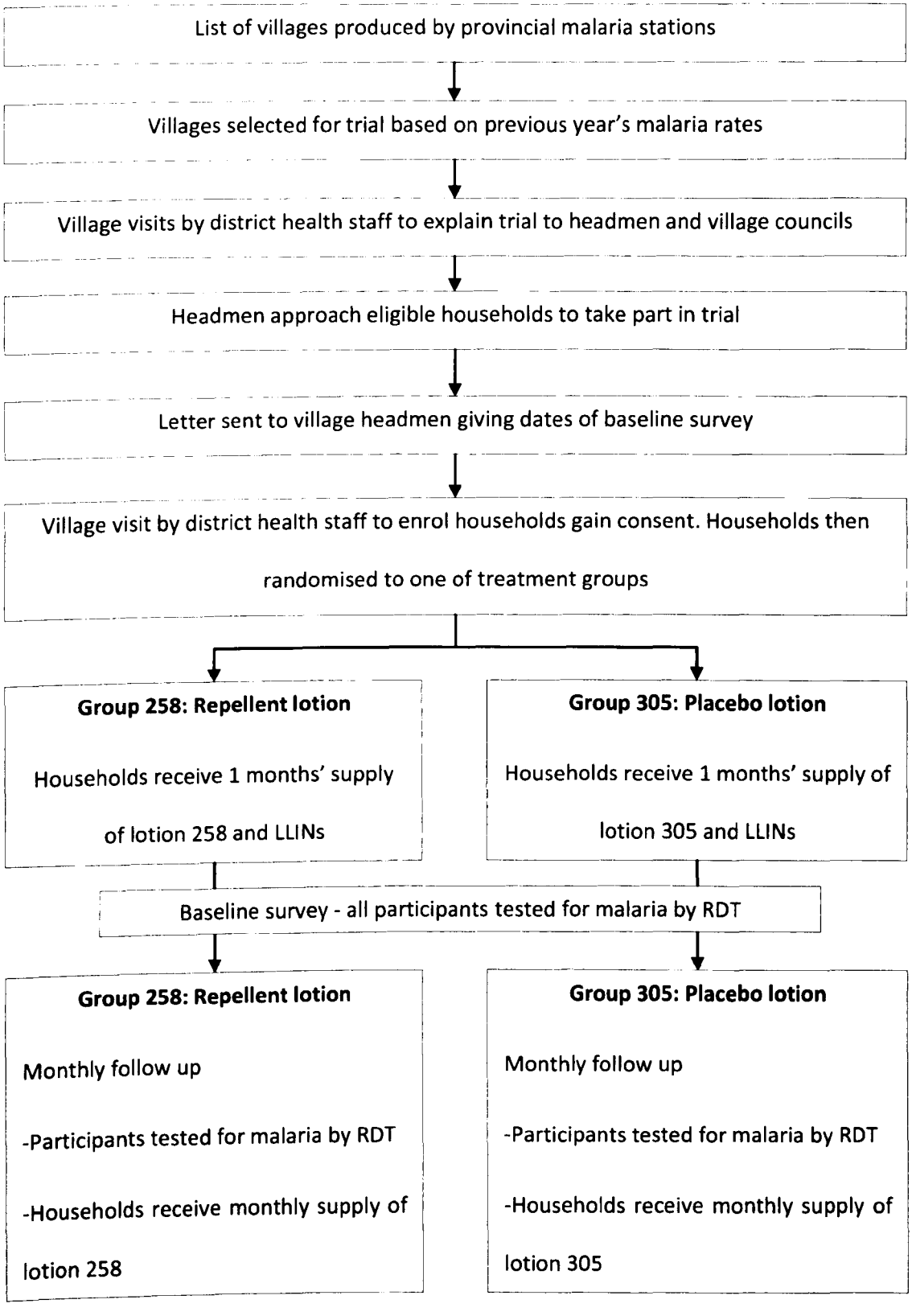


Figure 5.1 Flow chart of the process of recruitment and the planned progress of households through the trial.

Recruitment

Households were recruited through the village council who were consulted approximately a week prior to the baseline survey. Eligible households needed five volunteers aged 6-60 years. The village council identified potential households who then attended a meeting with district health staff where the study was described in full with opportunity for questions. Individuals that wished to participate also received this information in writing and signed a consent form to confirm that they understood the trial, including the possibility that they might receive the placebo lotion (Appendix A).

Baseline survey

The baseline survey was carried out at the same time as recruitment. The questionnaire collected basic information on wealth indicators, current malaria prevention behaviour for the household, and individual information on malaria exposure (Appendix B).

Follow-up surveys

All participants were tested by rapid diagnostic test (RDT) every month during active case detection. Follow-up surveys finished in December in both years to ensure testing throughout the wet season and into the following transition/dry season when previous surveys had found high parasite rates [44]. The RDTs used were CareStart™ Malaria Pf/Pv Combo test (AccessBio, NJ) which detects HRP2 antigen and pLDH and are able to detect both *P. falciparum* and *P. vivax* infections [164]. Follow-up surveys were conducted by field teams made up of village health workers (VHWs) and district health staff. The field teams gave the village council a list of the follow-up dates at the beginning of the trial and these were confirmed one week in advance. The village council ensured all households were present in the village on the day of follow-up. This process was necessary as many people do not live in their village houses during the wet season. If rice fields are a long distance from the village, households have another house next to their fields where the family lives during the rice planting and harvesting seasons. Participants who were missing on village follow-up visits would then be contacted by

VHWs who if necessary would visit that participant to carry out the RDT test. If an RDT could not be carried out within a week of the village follow-up visit the participant was deemed lost to follow-up. All positive cases were referred for immediate treatment following local guidelines through the district health teams working on the study.

Access to remote communities was anticipated to be a major challenge for data collection, particularly during the rainy season. The use of VHWs who are local community members with a few weeks of medical training, enabled data collection to continue throughout these months. The monthly survey was designed to be as simple as possible to enable VHWs who sometimes have limited education to complete the form. A simple one-page questionnaire collected data on LLIN use, repellent use and time spent away from the village (Appendix B). This visit was also used to provide the next month's supply of lotion and record the amount of lotion returned. District health staff rather than VHWs carried out the exit survey during the final month of data collection, to assess the acceptability of the repellent lotion in more detail.

Potential risk to participants

Finger-prick blood spots can cause modest, brief discomfort but are commonly used in this area and it is likely most individuals have already experienced their use. Risk from this procedure is very low and antiseptic wipes were used to prevent infection.

Use of repellent is universal worldwide and presents an extremely low risk to the user [94]. The DEET formulation used was a commercial brand made, registered and sold in the USA by SC Johnson Inc. The lowest effective dose of active 15% DEET (Chapter 2) was used, in a gentle aqueous lotion formulation to minimise risk and discomfort.

Repellent use may divert mosquitoes to non-users increasing their disease risk [121]. Therefore to protect both the placebo users and non-participants in the study villages a maximum of 25% of any village were recruited to the trial.

Only the consent forms contained personal information (names) and these were stored for the length of the trial in a locked filing cabinet in the project office in Vientiane. Number codes were used to identify participants during baseline and monthly surveys. Data was entered into a password protected Microsoft Access database.

5.3.2 Changes to trial design

During the first year of the trial participants also provided bloodspots at baseline and exit intended for serological analysis for dengue, Japanese encephalitis and typhus antibodies. Lower than expected malaria rates during the first year of the trial led to a revision of the sample size required. Therefore 1300 households were recruited in the second year rather than the previously planned 700. This increase meant the sideline serological work on arbovirus infections had to be dropped due to insufficient funding, and the trial focused solely on malaria infections the major vector borne disease in the area.

5.3.3 Participants

Participants were recruited from primarily rural agricultural workers that often work and sleep overnight away from the village during the wet season. Participants were aged 6-60 years old and households had to enrol at least five eligible participants to enter the trial. Study households also had to be separated by at least 10m to prevent diversion of biting from repellent users to placebo users [121].

5.3.4 Study setting

Households were recruited from 126 villages; 72 in Attapeu Province and 54 in Sekong Province. These are the most south-eastern provinces in the Lao PDR, sharing borders with Vietnam and Cambodia. The Annamite mountains run along the eastern Vietnam border and 60% of Attapeu Province is classed as mountainous [42]. The mountainous areas contain dense rainforest, contrasting with the open canopy dry forest on the plains. River valleys are the most important areas in both provinces for agricultural production, although the regular floods

that increase soil fertility can also destroy crops (mostly rice with the addition of maize for animal feed in some areas). The wet season is usually from April to October, followed by a cool dry season from November to January and a hot dry season from February to March. Rice farming is the main economic activity in these two provinces; 57% of Attapeu's population are farmers and in Sekong the figure is even higher at 71% [43].

The malaria situation in the Lao PDR is similar to that across the GMS; low overall, but a severe problem in forested border areas [18]. Within the Lao PDR, it is the southern provinces that are most affected by malaria, with *P. falciparum* parasite rate in Attapeu and Sekong about twice as high as the national average [43, 48]. *Plasmodium falciparum* is found in about 80% of cases, and *P. vivax* in most of the rest [44, 47, 142, 171]. The most important malaria vectors are *An. dirus*, *An. minimus* and *An. maculatus* [49, 50], and can all feed early and outside meaning they will be less affected by conventional control methods such as LLINs [44, 50, 51, 54, 56, 144, 146]. However although these behaviours may reduce the effectiveness of LLINs in reducing malaria transmission, non-use of a bed net is still highly associated with malaria in Lao PDR [44, 45]. Current policy in the Lao PDR is for the entire population at risk (estimated to be 70% of the country) to receive LLINs [162]. In addition free diagnosis and treatment with artemisinin combined therapy (ACT) has been implemented to poor populations. Resistance to artemisinin has not yet been detected in Laos [29].

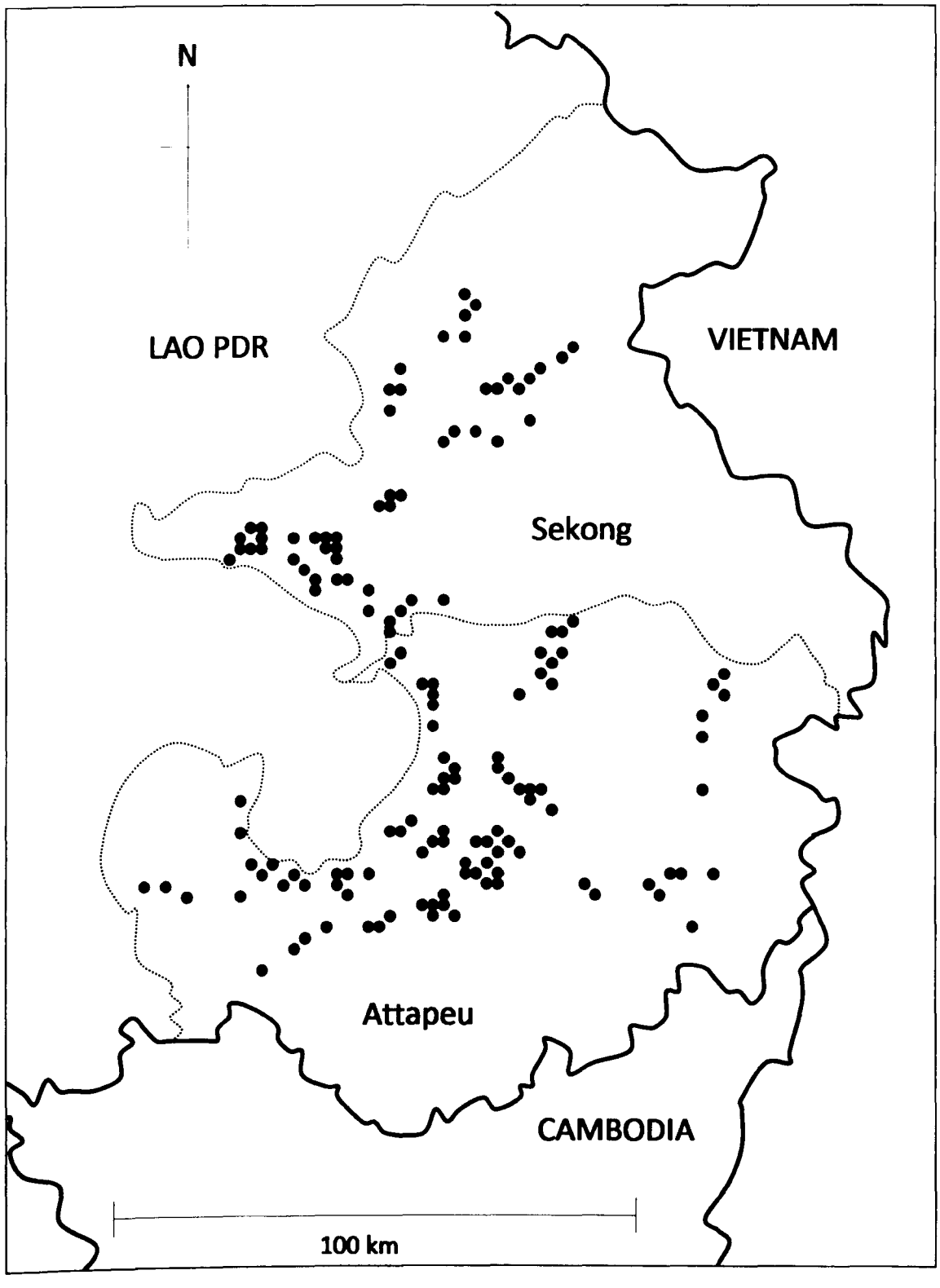


Figure 5.2. Location of study villages in Attapeu and Sekong provinces in Southern Lao PDR

5.3.5 Interventions

15% DEET or placebo lotion

A 15% DEET lotion was provided to half the households the other half of households received a placebo lotion (both supplied by SCJohnson, Racine, USA). Although previous repellent trials have used 20% DEET, a 15% DEET formulation was selected because this is the lowest concentration considered effective against Southeast Asian malaria vectors (Chapter 2, [124, 125, 138]). A low concentration was desirable to minimise the possibility of adverse events in study subjects as the trial would require them to use the repellent for up to nine months. Adult participants were provided with three 100ml bottles of lotion to last one month (approximately 10ml per day). Children under 12 years were provided with two bottles per month, corresponding to approximately 7ml per day. This amount was considered sufficient to apply the treatments to arms and legs as directed by trial staff. District health staff demonstrated the amount of lotion to apply to arms and legs during the recruitment process. Participants were instructed to use the lotion every evening. Full USA compliant consumer product information [94] were given verbally and in the local language. Any contraindications or side effects were recorded and reported at each monthly follow-up for appropriate action to local District Health departments.

Participants reported at each monthly follow-up visit how many evenings they had used the lotion. The amount of lotion returned was also recorded as a rough confirmation of the self-reported usage data. In addition random checks were carried out by trial staff: these involved visiting a village at dusk and smelling the arms of participants to check lotion had been applied.

Long-lasting insecticide treated nets

Study households were provided with sufficient LLINs (PermaNet®2.0, deltamethrin 55mg/m^2, mesh 25 holes/cm^2), defined as one net for every 1.5 persons in the household, plus another for use away from home. Participants were instructed to sleep under a net every night, particularly when away from the village. At monthly follow-up visits participants reported how

many nights they had slept under the nets in the village and also when sleeping in the forest or rice fields.

5.3.6 Outcomes

The primary outcome was household malaria incidence measured by monthly RDTs for *P. falciparum* and *P. vivax*. Positive RDTs, paired with a negative RDT matched by age and village, were confirmed by polymerase chain reaction [175] at the London School of Hygiene and Tropical Medicine. To avoid the risk of including relapse infections, only the first *P. vivax* positive result for each participant was recorded. If a participant tested positive for *P. falciparum* for two consecutive months the second result was also excluded in case of treatment failure.

5.3.7 Changes to outcomes

As mentioned above, at the start of the trial one of the secondary outcomes was changes in arbovirus antibodies in dried bloodspots, however they were dropped in the second year of the trial. All-cause fevers were also discarded during the interim analysis since these are not useful as proxy measure of malaria.

5.3.8 Sample size

The only reliable epidemiological data from the study area prior to the start of the trial was 32% prevalence of *P. falciparum* from village surveys during September 2008 (PSI Laos, unpublished data). Previous trials have shown an 80% reduction in clinical malaria due to *P. vivax* and a 44% reduction in *P. falciparum* infection [130, 131]. A 30-50% reduction in clinical malaria was therefore considered to represent a useful malaria intervention in this setting. An initial target of 500 households per arm was calculated to be able to detect a 50% reduction in clinical malaria associated with repellent use at 90% power and 95% significance. This was based on an estimated pre-trial incidence of 2-6%. However, a malaria incidence of only 0.7% was recorded in the first year of data collection. Therefore sample sizes were recalculated and

a sample size of 633 households per arm was found necessary to detect a 50% reduction in malaria incidence with 95% level of significance at 80% power. Less than 90% compliance with repellent and placebo lotion usage was around 26% in the first year so the sample size was adjusted to 798 households per arm, with 5 subjects in each household, to adjust for loss of power in the according to protocol analysis. With 1,500 participants (300 households) followed up over 5 months and a further 6,480 (1,296 households) followed up over 8 months, at least 74 malaria cases be would be required to measure a significant effect.

5.3.9 Interim analyses and stopping guidelines

The original sample size was based on weak survey data, so we recalculated the sample needed based on malaria incidence reported during the first year of the study. The trial manager was able to halt the trial at any point if the safety of participants was thought to be at risk, determined by any reports of severe reactions to or misuse of the lotions.

5.3.10 Randomisation

Households were randomised to one of the two treatment arms using equal groups allocation which was stratified by village. District health workers prepared labelled straws at a ratio of 1:1 for each village. The straws were picked by the head of household to assign treatment groups. This method was not perfectly executed as some heads of household returned their straws after picking a group meaning that the actual ratio between the treatment arms was not exactly 1:1.

5.3.11 Blinding

The repellent and placebo lotions were identified by 3-digit codes '258' or '305' as provided by the repellent manufacturer. Participants, field staff carrying out randomisation and follow-up surveys and trial staff performing data entry and analysis were blinded for the length of the trial. The repellent and placebo codes were only revealed after data entry and analysis was

complete. However, the possibility remains that participants were able to distinguish between the active repellent and the placebo by the effect on biting insects.

5.3.12 Similarity of interventions

The repellent and placebo were in identical bottles (Figure 5.3) and identified by a code on the label. Therefore there was potential for mix-ups to occur when participants received their bottles each month. For this reason the group codes were not only given to the heads of household on their ID cards, but the village council held a list as did the district health workers distributing lotions each month.

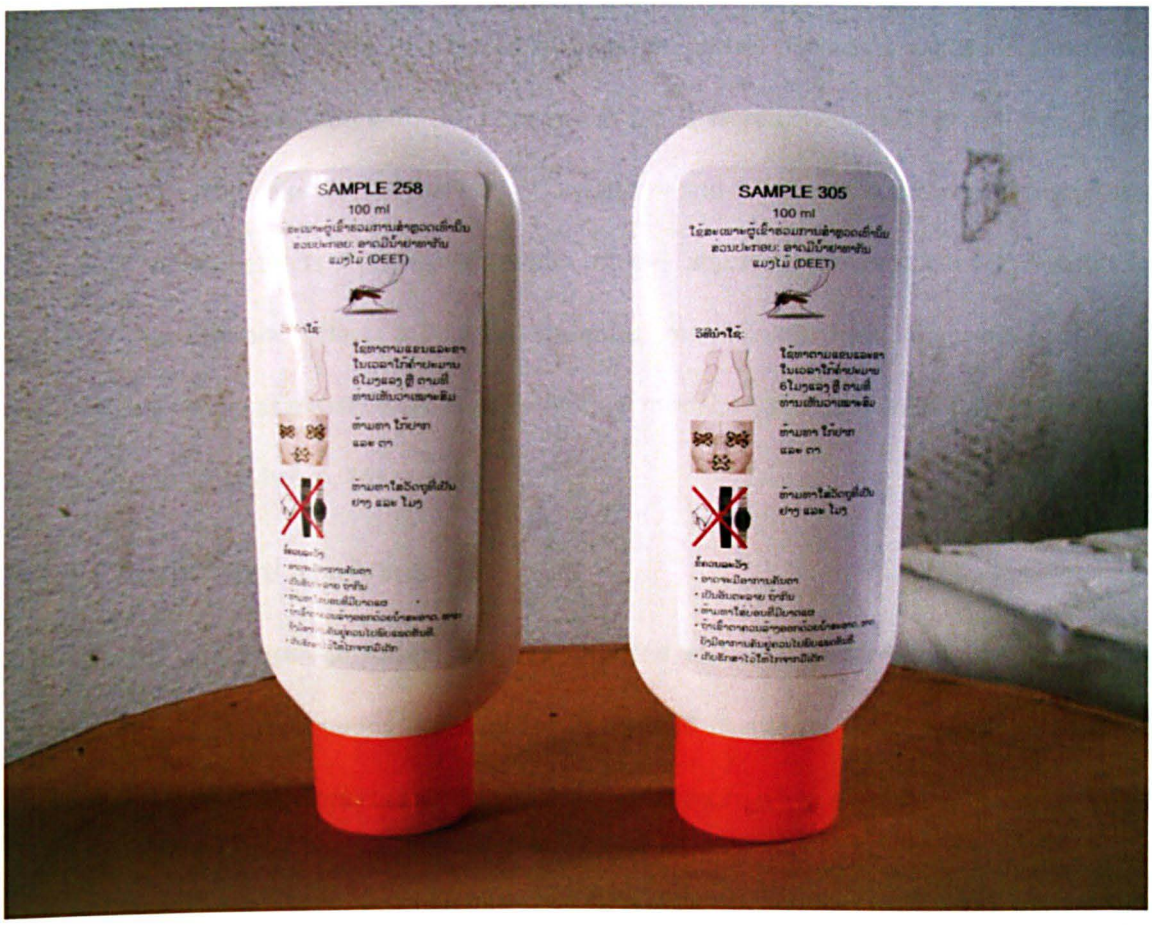


Figure 5.3. Bottles of placebo and 15% DEET lotions used in the trial.

5.3.13 Statistical methods

Analysis of this trial adhered as far as possible to a detailed analytical plan (Chapter 4) established before the investigators had access to the finalised data set. An initial intention-to-treat analysis was performed on all data, followed by a per protocol analysis which excluded participants who slept under an LLIN and used the lotion in the evenings less than 90%, 75% and 50% of the month. A principal component analysis (PCA) using data on education, house construction materials, type of electricity supply, ownership of motorbikes, tractors and televisions and animal ownership was carried out to develop overall socio-economic scores for each household [168]. The PCA scores along with age, gender, nights slept under an LLIN, and nights spent away from the village were considered for inclusion in the regression. All variables except treatment group were first tested by non-parametric univariate methods and those with a significant association with the outcome at $p<0.2$ were considered for inclusion in the final model. Outcomes of overall malaria, *P. falciparum* and *P. vivax* infections were tested in a multi-level mixed effects Poisson regression model adjusted for intra-cluster household variation by random effect methods. Variables maintaining their association in the multivariable model at $p<0.05$ were kept in the final model. All analyses were carried out using STATA version 12 (Statcorp, Texas, USA).

5.3.14 Additional analyses

Self-reported and observed lotion use, self-reported LLIN use and treatment group were entered into linear regressions adjusted for household clustering. Focus group discussions (chapter 6) suggested that participants used their lotion more when away from the village, so a pairwise-correlation was used to examine the relationship between nights spent away from the village and lotion use. Reports of adverse events were compared between treatment groups using a chi-squared test. A logistic regression was run to examine treatment group, age and gender as predictors for the reports of allergic reactions. The effect of LLIN use on malaria was estimated by comparison of malaria rates before and after baseline.

5.4 Results

5.4.1 Baseline data

The first round of recruitment from 25th June to 4th July 2009 enrolled 300 households to the trial and a further 1,297 households were recruited between 24th April and 18th May 2010. A total of 7,980 participants were initially recruited but 40 (0.5%) were excluded after the baseline survey as they were outside the 6-60 years age limit. Almost half of households (795, 49.8%) were randomised to the repellent arm and the remaining 802 allocated to the placebo lotion.

The individual level baseline data were very similar between treatment groups indicating successful randomisation. Overall slightly more women were recruited than men, but this did not differ between the treatment arms (Table 5.1). The age structures of the two treatment arms were also similar (Table 5.1). Ethnicity was similar between treatment arms (Table 5.1). Participants came from 16 different ethnic groups, although no data were available for 19% of participants approximately 12% of those who gave information were from the ethnic majority Lao who make up about 66% of the national population. A further 22% were from six groups within the Katuic ethno-linguistic family and 66% came from nine groups within the Bahnaric-Khmer ethno-linguistic family (Full data in Appendix E).

Table 5.1. Participant age, gender and ethnicities in each treatment arm.

Treatment arm		Repellent	Placebo
Total participants		3972	4008
Female		2186 (55.3%)	2187 (54.9%)
Median age (IQR)		19 (11-35)	20 (11-35)
Ethnicity:	Lao	396 (12.1%)	396 (12.3%)
	Katuic	712 (21.8%)	726 (22.5%)
	Bahnaric-Khmer	2154 (66.0%)	2106 (65.2%)

Baseline household-level socioeconomic data on occupation and education of household heads, house construction, possessions and animals were all examined individually before being combined using a principal components analysis (PCA). Over 95% of all heads of household were farmers (Table 5.2). Although this result was expected given that the trial aimed to recruit agricultural workers, the homogeneity of the variable made it unsuitable to include in the PCA. About a quarter of household heads had never received any formal education, about 60% had attended school for 1-5 years and less than 4% had received over 9 years of schooling. About two thirds of houses were made from wood rather than bamboo. Wooden stilts allow the house to be higher, which is generally an indication of greater wealth. A similar proportion had also managed to erect a metal roof over at least some part of their house, again an indicator of greater wealth. Sixty percent of households had no electricity supply and about half had access to a pump for water. Motorbike ownership was most frequent amongst the indicators used (televisions, radios, tractors and motorbikes), with about 40% of households owning at least one. About 60% of households also owned buffalo or pigs.

Along with occupation, cooking fuel, radio ownership and goat ownership were excluded from the PCA as they did not contain sufficient variation between the trial households. The PCA

therefore included the years of education of the head of household, housing materials, electricity and water supply, ownership of motorbikes, televisions and tractors, pre-trial ownership of bed nets and ownership of buffalo, pigs, chickens and dogs.

Using the eigenvectors and standardised variables, two scores for each household were created, PCA1 and PCA2. PCA1 was created from almost all variables and provided a general socio-economic score. PCA2 was more heavily influenced by animal ownership. Due to missing data PCA scores could only be created for 1,402 (87.8%) households. Non-parametric comparison of these scores between the treatment groups showed no differences (PCA1: p=0.602; PCA2: p=0.699; Table 5.2).

Baseline malaria rates were slightly higher in the placebo group, but not significantly so (Prevalence ratio: 0.82, 95% C.I. 0.5-1.37, p=0.454). Men had a slightly higher rate of malaria, but this did not reach significance (parasite rate in females: 0.61%, in males: 0.83%, p=0.263). Children aged 6-10 years were about twice as likely to have malaria as any other age group although the difference was only significant when compared to adults aged 31-40 years (Table 5.3).

Overall, the households in the two treatment groups were very similar despite wide variation between household, illustrating the success of the randomisation procedure.

Table 5.2 Household socio-economic data and resulting PCA scores per treatment arm.

Socio-economic variable		Repellent (n = 795)	Placebo (n = 802)
Heads of household:	% gave main occupation as "farmer"	97.0	95. 9
	Median (IQR) years of education	3 (1-5)	3 (1-5)
Houses:	% constructed from bamboo	34.3	35.5
	% constructed from wood	66.3	66.8
	% with a metal roof	68.6	68.7
Household services:	% with no electricity supply	59.0	61.7
	% with access to a water pump	51.6	52.1
	% using wood as main cooking fuel	97.5	97.0
Household possessions:	% that own at least one motorbike	38.7	41.3
	% that own a tractor	16.2	18.8
	% that own a radio	19.8	19.7
	% that own a television	23.2	24.3
	% that owned ITNs prior to the trial	86.3	85.5
Animals:	% owning buffalo	56.7	58.4
	% owning pigs	57.4	60.1
	% owning goats	11.2	11.4
	% owning chickens	70.4	73.4
	% owning dogs	47.2	46.3
Median PCA1 score (IQR)		-0.23 (-1.14, 1.09)	-0.23 (-1.23, 1.24)
Median PCA2 score (IQR)		-0.07 (-0.78, 0.66)	-0.07 (-0.89, 0.80)

Table 5.3 Baseline parasite rates per treatment arm with prevalence ratio and p-values to compare arms.

		Repellent	Placebo
Number of malaria cases		27	33
	P. falciparum	21	27
	P. vivax	4	6
	Mixed infections	2	0
Number of participants		3956	3984
Parasite rate (%)		0.68	0.83
Prevalence ratio (95% C.I.)		0.82 (0.5-1.37), p=0.454	

Table 5.4 Baseline parasite rates by age, children aged 6-10 years used as comparison group.

Age group (years)	Number	Total malaria cases	Prevalence (%)	Prevalence ratio (95% C.I.)	p
6-10	1859	25	1.34	-	-
11-15	1447	10	0.69	0.51 (0.25-1.07)	0.103
16-20	879	6	0.68	0.51 (0.19-1.39)	0.190
21-30	1340	9	0.67	0.50 (0.18-1.40)	0.119
31-40	1084	3	0.28	0.21 (0.06-0.76)	0.015
41-50	798	6	0.75	0.56 (0.14-2.23)	0.270
51-60	533	1	0.19	0.14 (0.02-1.16)	0.064
Total	7940	60	0.76	-	-

5.4.2 Trial Progress

Follow-up visits were carried out every month, finishing in December both years. Table 5.5 shows the number of households and participants surveyed at each monthly follow-up visit. No follow-ups were carried out in September 2009 due to widespread flooding in the study area, although households did receive replacement lotion as necessary. Therefore there were a potential 59,023 person-months, a loss to follow-up of 14.2% with no difference between treatment arms (repellent users 14.2%, placebo users 14.1%). A further 11.3% of participant-months were excluded from the intention to treat analysis due to incomplete data. Therefore in total 1,398 households and 44,024 person-months at risk (PMAR) entered the intention to treat analysis (Table 5.6 and Figure 5.4).

Table 5.5 Attendance of households and participants to follow-up surveys throughout the trial.

		Month								
		0	1	2	3	4	5	6	7*	8*
Repellent Households	Surveyed	795	776	740	645	673	719	659	545	371
	Missing	0	19	55	150	122	76	136	101	275
Placebo Households	Surveyed	802	779	753	657	677	738	654	546	377
	Missing	0	23	49	145	125	64	148	105	274
Repellent Participants	Surveyed	3972	3893	3776	3148	3304	3555	3158	2705	1721
	Missing	0	79	196	824	668	417	814	522	1506
Placebo Participants	Surveyed	4008	3890	3824	3201	3314	3659	3107	2696	1718
	Missing	0	118	184	807	694	349	901	558	1536

* 300 households were surveyed in 2009 for 6 months after baseline and 1,297 households were follow-up during 2010 for 8 months after baseline, giving a total of 1,597 households for months 0-6 and 1,297 households for months 7 and 8.

Table 5.6 Person-months at risk entering intention to treat analyses.

	Repellent	Placebo
Participants	3,972	4,008
Excluded by age	16	24
Person-months at risk	29,413	29,610
Person-months lost to follow-up	4,184 (14.2%)	4,170 (14.1%)
Person-months excluded by incomplete data	3,084 (10.49.9%)	3,561 (12.0%)
Person-months entering ITT	22,145 (75.3%)	21,879 (73.9%)

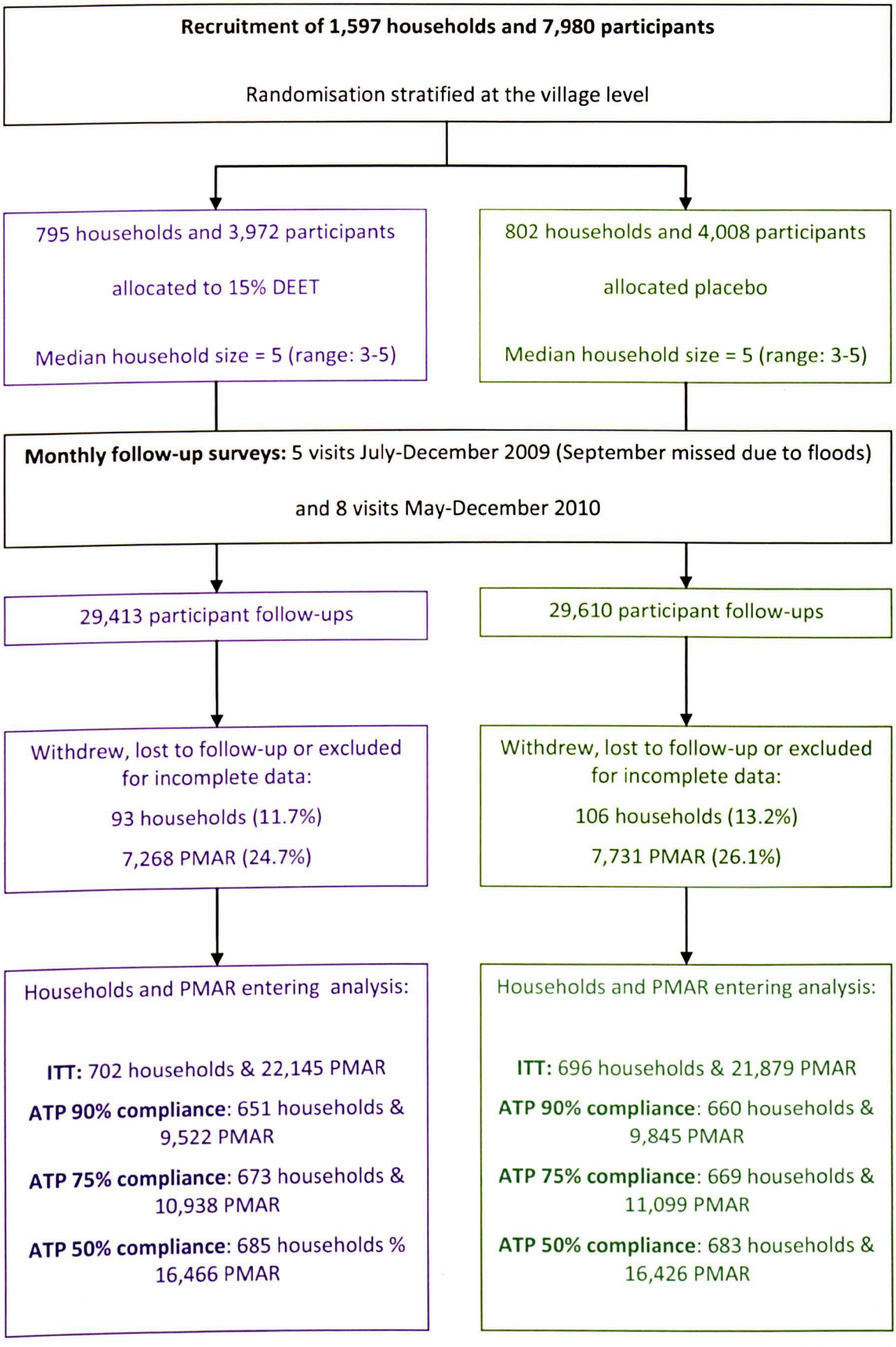


Figure 5.4 Progress of households from recruitment to analysis.

5.4.3 Intention to treat analysis

During follow-up there were 47 *P. falciparum* cases, 16 *P. vivax* cases and 11 mixed infections from 44,024 participant-months (Table 5.6). No cases were excluded due to concurrent positive results within a month. Our sample size calculation required 633 households per arm with an annual parasite rate of 0.015 to measure a 50% intervention effect at 95% significance with 80% power. Despite the drop in malaria rates following baseline the trial actually recorded an annual parasite rate of 0.020. The loss to follow up resulted in 696 households in the placebo arms and 702 households in the treatment arm. Therefore the trial was sufficiently powered to detect an intervention effect of at least 50%.

Table 5.7 Individual and household malaria incidence by treatment group.

	Repellent	Placebo
Person-months at risk (PMAR)	22,145	21,879
Malaria cases	36	38
P. falciparum cases	22	25
P. vivax cases	7	9
P. falciparum & *P. vivax*	7	4
Incidence	0.002	0.002

After exclusions for missing data a total of 44,024 person-months were entered into a multi-level mixed effects Poisson regression to account both for repeated measures over time and household clustering. Age, LLIN use and nights spent away from the village were not included in any models as they were not significantly related to the outcomes in univariate analysis (full results in Appendix D). The second socio-economic score, PCA2, was also dropped from the final models as it showed no effect. After accounting for socio-economic score and gender there was no difference between the placebo and repellent arms for overall malaria incidence (rate ratio 0.96, 95% CI 0.54-1.71, p=0.886, Table 5.7), *P. falciparum* incidence (rate ratio 0.86,

95% CI 0.45-1.67, p=0.665) or *P. vivax* (rate ratio 0.91, 95% CI 0.33-1.47, p=0.0.850). The models showed that a higher PCA1 score was associated with a reduced malaria risk in all models. Being female decreased the risk of overall malaria infection and *P. falciparum* infection by 40-50% (overall malaria rate ratio 0.62, 95% CI 0.39-1.00, p=0.051, *P. falciparum* rate ratio 0.54, 95% CI 0.31-0.92, p=0.025).

Table 5.8 Incidence rate ratios and 95% confidence intervals from ITT Poisson regressions on counts of overall malaria and *P. falciparum* infections.

Variable	Overall malaria	*P. falciparum*	*P. vivax*
Placebo	1	1	1
Repellent	0.96 (0.54-1.71 p=0.886	0.86 (0.45-1.67) p=0.665	0.91 (0.33-2.47) p=0.850
PCA1	0.76 (0.62-0.92) p=0.006	0.76 (0.60-0.96) p=0.016	0.71 (0.49-1.02) p=0.65
Male	1	1	-
Female	0.62 (0.39-1.00) p=0.051	0.54 (0.31-0.92) p=0.025	-

5.4.4 According to protocol analysis

A primary according to protocol (ATP) analysis excluded participant outcomes with less than 90% compliance (self-reported lotion and LLIN use <27 evenings per month and volume of lotion used <270ml per month). Secondary analyses were also run using compliance cut-offs of 75% (self-reported lotion and LLIN use <22 evenings per month and volume of lotion used <225ml per month) and 50% (self-reported lotion and LLIN use <15 evenings per month and volume of lotion used <150ml per month). This resulted in the exclusion of 25-56% of participant months (Table 5.8).

Table 5.9 Participants-months entering ATP analysis.

Compliance cut-off	Repellent	Placebo	Total
0% (ITT)	22,145	21,879	44,024
50%	16,466 (74.4%)	16,426 (75.1%)	32,892 (74.7%)
75%	10,938 (49.4%)	11,099 (50.7%)	22,037 (50.1%)
90%	9,522 (43.0%)	9,845 (45.0%)	19,367 (44.0%)

Age, gender, net use and nights spent away from the village were not included in any models as they were not significantly related to the outcome by univariate analysis (see Appendix D). The results of the ATP analyses were in line with the outcomes of the ITT analysis. After accounting for socio-economic scores there was no difference between the placebo and repellent arms in the overall malaria, *P. falciparum* or *P. vivax* models (Tables 5.9-5.11). PCA1 was again associated with a decrease in malaria risk and PCA2 was associated with an increased risk of *P. falciparum* infections.

Table 5.10 Incidence rate ratios (95% confidence intervals) and p-values for predictors in Poisson regressions for overall malaria incidence excluding participants with lower than 50%, 75% and 90% compliance.

	Compliance		
	>50%	>75%	>90%
Placebo	1	1	1
Repellent	1.13 (0.55-2.32) p=0.730	1.36 (0.53-3.51) p=0.525	1.45 (0.53-3.99) p=0.467
PCA1	0.79 (0.63-1.00) p=0.048	0.55 (0.37-0.82) p=0.004	0.58 (0.38-0.88) p=0.011
PCA2	1.41 (1.05-1.91) p=0.025	-	-

Table 5.11 Incidence rate ratios (95% confidence intervals) and p-values for predictors in Poisson regressions for *P. falciparum* incidence excluding participants with lower than 50%, 75% and 90% compliance.

	Compliance		
	>50%	>75%	>90%
Placebo	1	1	1
Repellent	0.96 (0.43-2.16) p=0.920	1.25 (0.38-4.13) p=0.710	1.56 (0.45-5.39) p=0.482
PCA1	-	0.57 (0.36-0.91) p=0.017	0.56 (0.34-0.92) p=0.024
PCA2	1.51 (1.12-2.04) p=0.007	2.00 (1.18-3.41) p=0.010	1.99 (1.12-3.53) p=0.019

Table 5.12 Incidence rate ratios (95% confidence intervals) and p-values for predictors in Poisson regressions for *P. vivax* incidence excluding participants with lower than 50% and 75% compliance.

	Compliance		
	>50%	>75%	>90%
Placebo	1	1	1
Repellent	1.01 (0.32-3.16)	1.92 (0.48-7.68)	1.65 (0.39-6.90)
	p=0.993	p=0.356	p=0.494
PCA1	0.65 (0.42-1.02)	0.51 (0.28-0.94)	0.61 (0.33-1.11)
	p=0.062	p=0.031	p=0.105

5.4.5 Compliance

Participants aged over 12 years were provided with 300ml of lotion per month and median volume of lotion used was 260ml (IQR: 200-300ml). Children under 12 years were provided with 200ml per month and median volume used was 160ml (IQR: 133-200ml). Almost half of participants had used all lotion supplied each month and about 60% self-reported full compliance with daily lotion use.

Although compliance and malaria rates were broadly similar in the two treatment arms, a closer examination showed some differences. After adjusting for household clustering, higher observed compliance was significantly associated with a lower odds of malaria in both treatment arms (placebo group odds ratio: 0.14, 95% CI 0.05-0.41, p<0.001; repellent group odds ratio 0.27, 95% CI 0.10-0.77, p=0.015). Figure 5.5 shows malaria rates decline with increasing compliance in the repellent group. In the placebo group the relationship is similar between compliance and malaria rates, but is less clear cut.

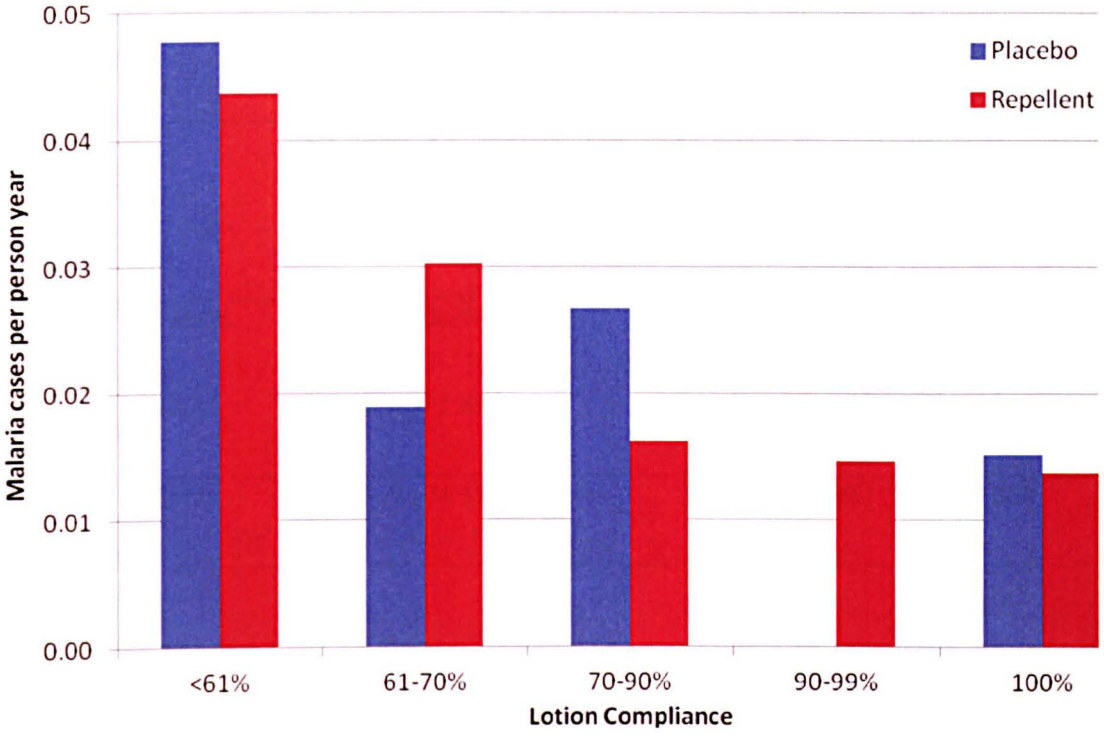


Figure 5.5 Malaria incidence in different repellent and placebo-use groups.

A linear regression accounting for household clustering showed a weak but statistically significant relationship between observed and reported lotion use ($r^2=0.194$, $p<0.001$). Compliance with LLIN use was much higher than lotion use regardless of treatment group (repellent users 97.0%, placebo users 97.4%, $p=0.547$). Linear regression showed a significant relationship between compliance to the two types of interventions ($p<0.001$). As shown in Table 5.13, participants who were non-compliant with LLIN use were more likely to be non-compliant with lotion use (logistic regression accounting for household clustering, $p<0.001$).

Table 5.13 Relationship between compliance with LLINs and compliance with lotions, compliance with lotion defined as both self-reported and observed lotion use of 100%.

		Repellent or placebo lotion use		
		<100%	100%	Total
LLIN use	<100%	1,280 (90.0%)	142 (10.0%)	1,422
	100%	36,264 (73.6%)	12,983 (26.4%)	49,247
	Total	37,544 (74.1%)	13,125 (25.9%)	50,669

Travel away from the village was more common in men (males 43.0%, females 38.8%, χ^2=95.6, p<0.001) and during September (Figure 5.5). However no correlation was found between increased time away and increased lotion use (r^2=0.002).

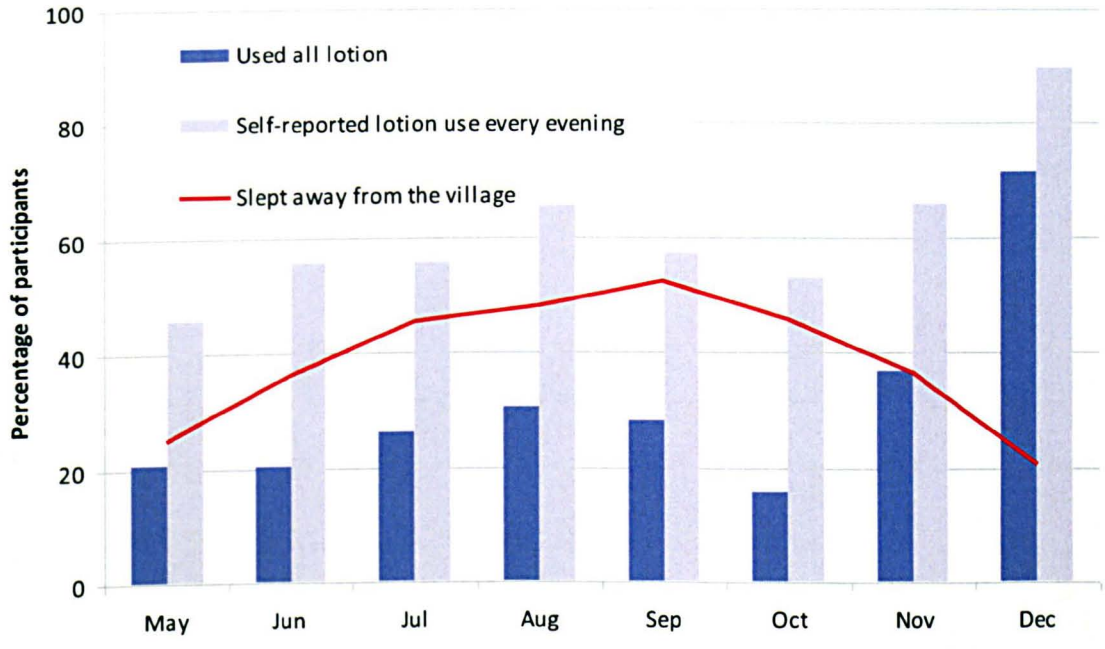


Figure 5.6. Percentage of participants each month who slept away from the village each month with corresponding lotion use.

5.4.6 Adverse Events

Approximately one in ten participants reported one or more allergic reactions during the trial (repellent users 10.9%, placebo users 10.1%). A logistic regression accounting for household level clustering indicated reports of allergies were not associated with treatment group ($p=0.518$). However, women were about 30% more likely to report an adverse reaction than men (odds ratio=1.38, $p<0.001$) and there was no effect from age on the likelihood of allergies ($p=0.101$).

5.4.7 Long-lasting insecticide nets

All households in both treatment arms were provided with LLINs at baseline. There was an overall reduction of 73.8% (95% C.I: 44.3-103.4%) in malaria prevalence between baseline and the first month of follow-up. This effect persisted throughout the trial with malaria rates between 52-90% lower than baseline and low prevalences recorded over the peak of the wet season from June to September (Figure 5.6).

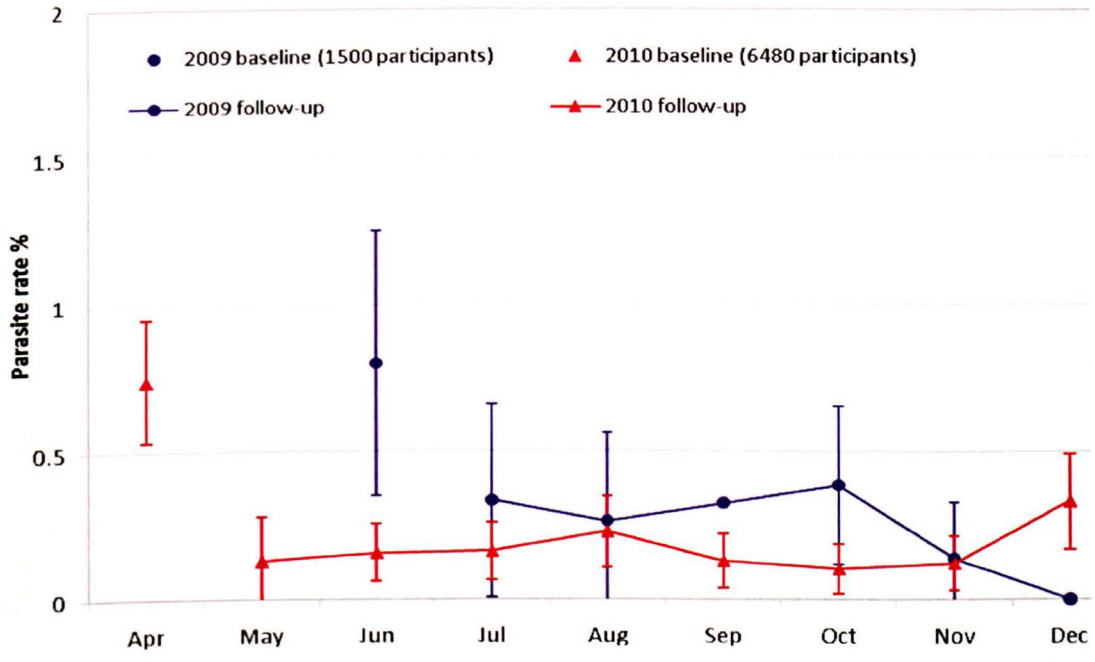


Figure 5.7. Monthly parasite rates at baseline and follow-up during 2009 and 2010.

5.5 Discussion

5.5.1 Summary

A randomised placebo controlled trial of 15% DEET repellent lotion used by agricultural communities in southern Lao PDR was carried out over two wet seasons in 2009-2010. The trial was powered to detect an intervention effect of 50% on malaria incidence. No significant reduction in malaria incidence was found from the use of the topical repellent.

The regression analysis identified socio-economic scores as being the most important risk factors for malaria. The first score produce from the PCA was influenced mostly by housing and possessions. An increase of 1 in this score could represent having a tiled roof compared to a thatched one, ownership of 1.5 more motorbikes or 1 more television and corresponded with a 20-45% reduction in the risk of household malaria. Although malaria researchers in the Lao PDR have not previously looked for a link between malaria risk and wealth, our results are consistent with the findings of other studies where lower socio-economic status is associated with increased malaria risk [176, 177].

The second PCA component related to animal ownership and an increase of 1 in this score could represent owning 12 more buffalo or 10 more pigs, and also corresponded with an approximate doubling in the risk of *P. falciparum* malaria. This variable had less association with overall malaria and *P. vivax* incidence. Participants in households owning more animals may spend more time outside and experience a higher level of biting, raising their risk of *P. falciparum* malaria.

There was a sustained drop of over 50% in monthly malaria prevalence from baseline when all households were provided with LLINs. Although this effect could be a result of changes in malaria as there was no control group, the fact that lower prevalences continued to be recorded throughout the wet season when they would have been expected to increase does support the effect of the intervention. In addition the baseline rates in 2009 and 2010 were

similar suggesting there had been no overall drop in malaria rates in the area between the two years. In conclusion this trial shows no additional protection from malaria from topical repellent above that provided by LLINs the current best practice in the study area.

5.5.2 Limitations

The use of 15% DEET was chosen based on human landing catches in a village setting in rural Lao PDR (Chapter 2). This meant that the protection measured would accurately reflect the perception of biting pressure experienced by the participants of the trial. However the major malaria vectors in the area *An. minimus* and *An. maculatus* were very rare in entomological collections, so the level of protection by 15% DEET from these species was not tested. Anophelines show less response to repellents than other genera, including *Stegomyia* and *Culex* mosquitoes that made up the majority of catches in the local area [115, 118]. Therefore the recorded 98.9% protection against biting from15% DEET is potentially an overestimate for the protection from malaria vectors.

While this gap in the efficacy testing of the intervention should be acknowledged, it is probably not as important as the variation in the dosage of DEET applied to the skin that would result from variation between user applications. A participant applying only 5ml of the repellent lotion, would achieve the same DEET dosage as 10ml of a 7.5% DEET lotion. Even two participants applying the same volume of lotion would end up with different dosages depending on their relative body size. This variability is a major limitation with topical insect repellents as an intervention tool, but this does not rule out other forms of repellent such as impregnated fabrics that can be better standardised.

The trial was double-blinded and unblinding was only carried out after data analysis was complete. Landing catches in a village setting found a reduction in mosquito biting of over 95% from the trial repellent compared to the placebo indicating repellent users might easily be able to distinguish which group they had been assigned to after a short period of use. All households in one treatment arm from a particular village withdrew from the trial after three

months because they believed they had the placebo rather than repellent lotion, and unblinding found that they were correct. This meant the blinding while as good as it could be was not perfect. An alternative that could prevent the two lotions being directly compared would be to assign separate batch numbers to each household's supply meaning there was no easy way for participants to separate the two treatment groups. This method was not implemented in this trial as distribution of lotion was made each month by village health workers with limited education and it was judged that the system should work as simply as possible to avoid confusion.

In order to avoid artificially increasing malaria rates in the placebo group through diversion of biting from repellent users, a maximum of 25% of any village were recruited to the trial. However although all members of a household were randomised to the same arm, there was no way to enforce repellent use by all members of a household at all times. Therefore, diversion of mosquitoes from participants using the repellent to participants within the same household not using the repellent could increase malaria risk for those individuals. Individual compliance could have a large impact on this household randomised trial, and the ability to accurately measure this could also have an important impact on the outcome. Participants were not compelled to use repellent during this trial, a demonstration of how much, where and when to apply the lotion was given at the start of the trial. Participants self-reported the number of evenings per month they used the lotion and as a second measure the amount of lotion returned was recorded. Random checks were also carried out in the evenings on a small sample of villages. Self-reported data on compliance is notoriously unreliable, so these data were combined with the volume of lotion used in order to filter non-compliers out of the ATP analysis. However uncertainty remained over the actual daily use of the lotion, in particular whether all members of a single household had used the lotion supplied equally.

Compliance was lower in this trial than in previous repellent trials. Self-reported and observed data gave estimates of full compliance from 48-60%, other trials have reported compliance

levels from 68-98% [129-131, 178]. Only one repellent trial, in Ecuador and Peru, reported compliance around 50%, but this was because not enough repellent had been provided [128]. A mathematical model has suggested that compliance would be the most important influence on the success of repellent interventions [137], so this low level of compliance may explain at least some of the lack of effect found in our results.

This trial was focused on agricultural populations and the results may not be applicable to one important malaria risk group within the GMS. Forestry workers spend much more time in the forest potentially without good access to healthcare, and live in more temporary accommodation meaning they may be more exposed to vector biting. They often come from elsewhere in the region and their movement between endemic and non-endemic areas has been linked to the spread of antimalarial resistance in the region [18].

5.5.3 Conclusions

Southern Lao PDR shares similarities in malaria vectors, environment and the human population with much of the GMS and the results of this trial are likely to be applicable across this region. Topical repellents are not likely to be a suitable intervention for agricultural populations in this region already using LLINs who require long-term protection throughout the wet season. The ITT and ATP analyses produced similar results meaning that non-use of the lotions is most likely not responsible for the lack of effect observed. But variability between individual dosages, inherent in the use of topical repellent may have confounded the results. In addition to this variability in dosage topical repellents require application every day relying on a large behaviour change for full protection. Therefore the longer the intervention needs to last, the lower the probability of full compliance. Therefore further work to test the effectiveness of repellents that can be better standardised, such as impregnated fabrics are recommended as a next step.

5.6 Additional Information

5.6.1 Trial Registration and Study Protocol

This trial is registered with ClinicalTrials.gov number NCT00938379. The full study protocol is described in Chapter 2.

5.6.2 Funding and Support

This trial is supported by the PSI Innovations Fund. Both the 15% DEET and the placebo formulations were donated by SC Johnson, who had no part in the design and analysis of this study.

5.6.3 Ethical Approval

Ethical Approval for this study was obtained from the Lao Ministry of Health and the Ethics Committee of the London School of Hygiene & Tropical Medicine (Appendix A).

Chapter 6: Attitudes of agricultural populations in southern Lao PDR to the use of topical insect repellent used to reduce malaria.

6.1. Abstract

A cluster randomised trial was started in southern Lao PDR in 2009 to determine whether the use of 15% DEET and long-lasting insecticidal nets (LLINs) could reduce malaria cases compared to those using a placebo and LLINs. Following the first year of the trial focus group discussions were held to establish the opinion of the repellent users to the lotions used and explore reasons for non-compliance. Questionnaires administered throughout the trial collected similar information. A comparison between self-reported lotion use and the amount of lotion returned to health staff was also made. Only 20.2% of placebo users complained that the lotion was failing to reduce biting, although this was significantly higher than repellent users (13.1% $p<0.001$). Repellent users were more likely to complain about the smell of the lotion (repellent users 49.5%, placebo users 44.1%, $p=0.003$) however this did not affect compliance (smell given as reason for non-compliance in repellent users 12.3%, placebo users 12.1%, $p=0.600$). A weak but consistent relationship was found between self-reported lotion use and the amount of lotion that was observed to have been used ($R^2=0.21$). An important finding from the FGDs was that many men and young children are outdoors fishing and hunting throughout the night, when it had previously been presumed people were protected by LLINs. Adult men also spent more time sleeping away from home in the forest, in contrast to their wives who spent time away from the village at the family's rice fields. Previous village-based studies in southern Lao PDR found no difference between malaria risk in men and women, which was in contrast to the pattern in the rest of Southeast Asia where adult men are usually at greatest risk. Our findings showed adult men in southern Laos are carrying out high risk behaviours making them more susceptible to malaria.

6.2. Background

Vector borne diseases are a significant problem in Southeast Asia where almost 700,000 disability-adjusted life years (DALYs) were lost to malaria, dengue and Japanese Encephalitis in 2004 [179]. Of these malaria currently causes most deaths, although within the Greater Mekong Sub-region (GMS) malaria cases fell by almost a quarter between 1998 and 2007 [18]. In this region malaria is a rural disease, disproportionately affecting poor and remote populations found along forested, mountainous border areas [18, 141]. In the Lao PDR, this means malaria is most prevalent in the south-east, where in the two provinces of Attapeu and Sekong, 90% of the population are rural and about half of villages have no road access during the wet season [43]. A risk analysis in Attapeu found that malaria cases were associated with sleeping without a net or under an untreated net, sleeping away from home, visiting the forest and living within 2km of a suspected mosquito breeding site [45].

Local malaria vectors are *Anopheles dirus*, *An. minimus* and *An. maculatus* with *An. dirus* likely to be the major vector [44, 49-51]. Members of the *An. dirus* complex are strongly associated with forest habitats which provide dry season larval sites allowing year round malaria transmission cycles to persist [52]. Biting behaviour in the *An. dirus* complex varies with sibling species, early evening feeding is observed in *An. cracens* and *An. scanloni* with others feeding later at night [54]. It is not yet clear which sibling species are present in southern Lao PDR, to date sibling identification has only been successfully carried out in one central-Laos site which found *An. dirus* s.s. [52]. *An. minimus* is also recorded biting in the early evening across Southeast Asia [55, 145, 180]. All three bite outdoors as well as indoors, with only *An. maculatus* showing a marked preference for exophagy [44, 49, 50, 56, 143-147, 150, 151]. This is important since current malaria control policy in the Lao PDR is for the entire population at risk, estimated to be 70% of the country, to receive long-lasting insecticide treated nets (LLINs) [162]. LLINs are highly effective against mosquitoes indoors, and may reduce the overall vector density lowering both the indoor and outdoor biting populations. Nevertheless, where vectors

bite outdoors there is a need for additional measures of protection. A trial was designed to determine whether mosquito repellent applied daily in the early evening could protect against malaria vectors that bite outdoors in the early evening before subjects were asleep under LLINs (Chapter 3).

The present study aimed to find out more about the attitudes of the local people to insect repellent. The main aim was to identify any problems users experienced with the repellent and to explore reasons for non-compliance. Further issues concerning local people's perceived exposure to mosquitoes and their use of repellents or other methods of personal protection against mosquito biting outside the trial were also explored. A behaviour change like daily use of insect repellent would require the perception of biting or malaria to be an important problem. To examine the reliability of self reported data on lotion use a comparison was made between this and the amount of lotion left in the bottle as reported by health staff.

6.3. Methods

6.3.1. Questionnaires

Questionnaire surveys were administered to each household (300 households for 5 months and 1,297 households for 8 months = 11,876) every month they participated in the trial. Forms were written and designed in English and translated into Lao (Appendix B). Training and consultations with local health staff were then used to check the questions, multiple choice answers and translations before the start of the trial. These forms were simple as they were often completed by workers with a low level of literacy. Therefore most questions could be answered by numbers, where responses were in words, they were entered into the database in Lao and translated into English by an independent team. Compliance with lotion use was measured by self-reported evenings per month. As self-reported data are unreliable, health staff also estimated the amount of lotion participants had used each month. As health staff visited participants' villages for follow-up and were therefore unable to carry weighing scales

or other equipment, a scoring system was used to estimate the lotion returned. Three 100ml bottles were given to each participant each month and the amount of lotion left in each bottle was scored out of 5, giving a total score 0-15. Both measures were compared to determine the level of accuracy in the self-reported data. Participants were also asked about any problems they experienced using the repellent, such as itching or headaches.

6.3.2. Focus Group Discussions

Four focus group discussions (FGDs) each involving a 6-7 participants were carried out in Layaokao and Kasom villages in Samackixay District, Attapeu Province, the Lao PDR in April 2010. Participants were volunteers from each village who had taken part in the repellent trial the previous year aged 13-60 years. They were divided into two groups and separate discussions were held with each group. The first group were those who had reported good compliance with repellent use and the second group were made up of participants who had reported not using the repellent during the trial. Question lines were developed around problems with the repellent, where and when repellent was used, exposure to mosquito biting particularly any differences between age groups and genders and other forms of personal protection used. Discussions were carried out in the Lao language and run by a moderator who was fluent in both Lao and English, the trial manager (VCH) sat outside the discussion circle close to the moderator [181]. Discussions were also recorded on digital recorder. A Lao government observer and two local health staff were also present and made notes at each FGD.

6.3.3. Data Analysis

Analysis of FGD data started in the field. Following each session a discussion of the main themes that had emerged was held by trial staff and observers. The FGDs themselves were also reviewed to ensure all question lines had been addressed and that participants had not been lead into answering in a particular way. Following each discussion digital recordings and field notes were used to produce transcripts in English (Appendix). Discussions stopped when

both trial staff and observers agreed that no new information was being gathered. Transcripts were analysed according to the methods described by Dawson and Manderson [181]. Differences in compliance and opinions of the lotion between the two treatment groups were analysed using rate ratios. A correlation was performed between self-reported and health staff observed compliance using pairwise deletion of missing data. Data analysis was carried out in STATA 12 and all charts produced in Excel.

6.3.4. Ethical Considerations

Ethical approval for the repellent trial was provided by the London School of Hygiene & Tropical Medicine Research Ethics Committee and by the Lao Ministry of Health. The aim and procedure of the FGD were explained to participants, village elders and district health staff prior to the start of discussions. Participants were identified by codes to protect their identities.

6.4. Results

6.4.1. Study Population

Over the course of the intervention trial 10,513 household questionnaires (11.5% lost to follow-up) were administered to 1,597 households, involving a total of 7,980 participants (Table 6.1). There were no differences between the age structure and gender balance of each treatment arm (Chapter 5). The repellent trial needed to ensure ethnic minorities were included as these groups are disproportionately affected by malaria in Southeast Asia and Lao PDR [18, 41]. The Lao PDR is incredibly ethnically diverse and the Lao ethnic majority only make up 54.6% of the total population [43, 182]. Amongst trial participants Lao only made up 9.9% of households, the remainder coming from sixteen ethnic groups in the Katuic and Bahnaric-Khmer ethno-linguistic categories (Table 6.1). More than half of all trial households belonged to the four ethnic groups: Triang, Brao, Halak and Kriang which make up 1.6% of the

general population (full data in Appendix F). There were a total of 25 FGD participants, of which 14 (56%) were women, and the median age was 36 (IQR: 21-47).

Table 6.1 Age, gender and ethnicity of the study population

		Repellent	Placebo	Total
Participants (households) recruited		3,972 (795)	4,008 (802)	7,980 (1,597)
Household follow-up visits		5,913	5,963	11,876
Questionnaires returned		5,225 (88.4%)	5,288 (88.7%)	10,513 (88.5%)
Median age in years (IQR)		19 (11-35)	20 (11-35)	20 (11-35)
Female		2,193 (55.2%)	2,196 (54.8%)	4,389 (55.0%)
Ethno-linguistic category	Lao	396 (10.0%)	396 (9.9%)	792 (9.9%)
	Katuic	712 (17.9%)	726 (18.1%)	1,438 (18.0%)
	Bahnaric-Khmer	2,154 (54.2%)	2,106 (52.5%)	4,260 (53.4%)
	No or incomplete data	710 (17.9%)	780 (19.5%)	1,490 (18.7%)

6.4.2. Opinions and problems reported with trial lotions

Both lotions were well liked, over 90% of participants reported after 6-8 months that they enjoyed using the lotions, with no significant preference for either lotion (repellent users 92.5%, placebo users 91.8%, Table 6.2). Although only requested to use the lotion in the evening, almost all participants also applied the lotions voluntarily during the day as well (repellent users 96.9%, placebo users 96.7%) and about 40% did this every month throughout the trial (repellent users 40.2%, placebo users 38.2%).

Complaints about the lotions were most commonly to do with the smell (44.0%), followed about equally by no reduction in biting (16.5%), allergies (15.4%) and headaches (14.9%).

There were differences between the treatment arms as to the problems reported. After accounting for household clustering, it was found that there were no significant differences in reports of smell (p=0.314), allergic reactions (0.988) or headaches (0.565) between the two treatment groups (Table 6.2). However repellent users were significantly less likely to complain of mosquito bites whilst using the lotion (Table 6.2, p=0.002). Participants who did not use the lotion every evening were given a second opportunity to report problems with the lotion, when asked about their reasons for non-compliance. After accounting for household clustering, no differences between the two treatment groups were found in the reasons given for non-compliance (Table 6.3).

Age and gender were also included in the models and it was found that men were significantly less likely to report problems with the lotions (odds ratio: 0.79, 95% CI: 0.74-0.85, p<0.001). Women were more likely to report problems with the smell (odds ratio: 1.30, 95% CI: 1.18-1.44, p<0.001), allergies (odds ratio: 1.30, 95% CI: 1.12-1.52, p=0.001) and headaches (odds ratio: 1.39, 95% CI:1.20-1.61, p<0.001), although they were not any more likely than men to find the lotion had failed to repel mosquitoes (odds ratio: 0.95, 95% CI: 0.85-1.07, p=0.383). Age was also found to affect the reporting of problems, with older people complaining more frequently of the smell (p=0.035), allergies (p=0.001) and headaches (p<0.001), but age had no effect on the failure of the lotions to repel biting (p=0.368).

Problems with the smell also emerged from FGDs where some users found the fragrance overpowering to the point of headaches and nausea. Allergic reactions to the lotions were also reported in the FGDs, although this was sometimes linked to using the lotion contrary to directions for example getting it into wounds or the eyes. Others reported adverse effects after using large doses of the repellent, although one participant used it every day for cosmetic reasons believing it to soften the skin.

Table 6.2 Problems reported about the repellent and placebo lotions at monthly follow-up visits.

	Repellent users (%)	Placebo users (%)	Odds Ratio (95% C.I.)	P
No problems reported	90.4%	89.95%	1.05 (0.90-1.23)	0.540
Unpleasant smell	5.08	4.62	1.10 (0.91-1.34)	0.314
Allergy	1.70	1.70	1.00 (0.76-1.30)	0.988
Headache	1.58	1.71	0.93 (0.72-1.20)	0.565
Biting not reduced	1.37	2.26	0.60 (0.43-0.83)	0.002

6.4.3. Self-reported lotion use

Participants were asked to use their lotion every evening, and lotion use was assessed at the end of each month. Participants over 12 years were given 300ml of lotion per month, allowing 10ml per day for an adult. A single application of 10ml was used during landing catches (Chapter 4) and volunteers reported this volume to be more than enough with most having to reapply to already treated areas to use the full amount. Median lotion use was 86.7% per month, which equated to 260ml or 8.7ml per day. Approximately 60% of participants self-reported full compliance with lotion use each month with no difference between treatment arms (repellent users 61.3%, placebo users 62.2%, p=0.588). Health staff also observed the volume of lotion that was returned and found less than half of participants had used all the lotion (repellent users 47.4%, placebo users 48.1%). A comparison of full compliance from these two measures showed the false positive rate, self-reported full compliance with non-lotion use was 46.7%, much higher than the false negative rate, complete lotion use with self-reported non-compliance 28.5% (Table 6.3). The relationship between self-reported and

observed lotion use was very similar between the two treatment arms (Figure 6.1). A weak positive correlation was found between the two measures (r^2=0.19).

Table 6.3 Observed and self-reported lotion use per participant-month.

		Observed lotion use per month		
		<100%	100%	Total
Self-reported lotion use per month	<100%	14,122 (72.9%)	5,259 (27.1%)	19,381
	100%	12,339 (39.1%)	18,949 (60.6%)	31,288
Total		26,461 (52.2%)	24,208 (47.8%)	50,669

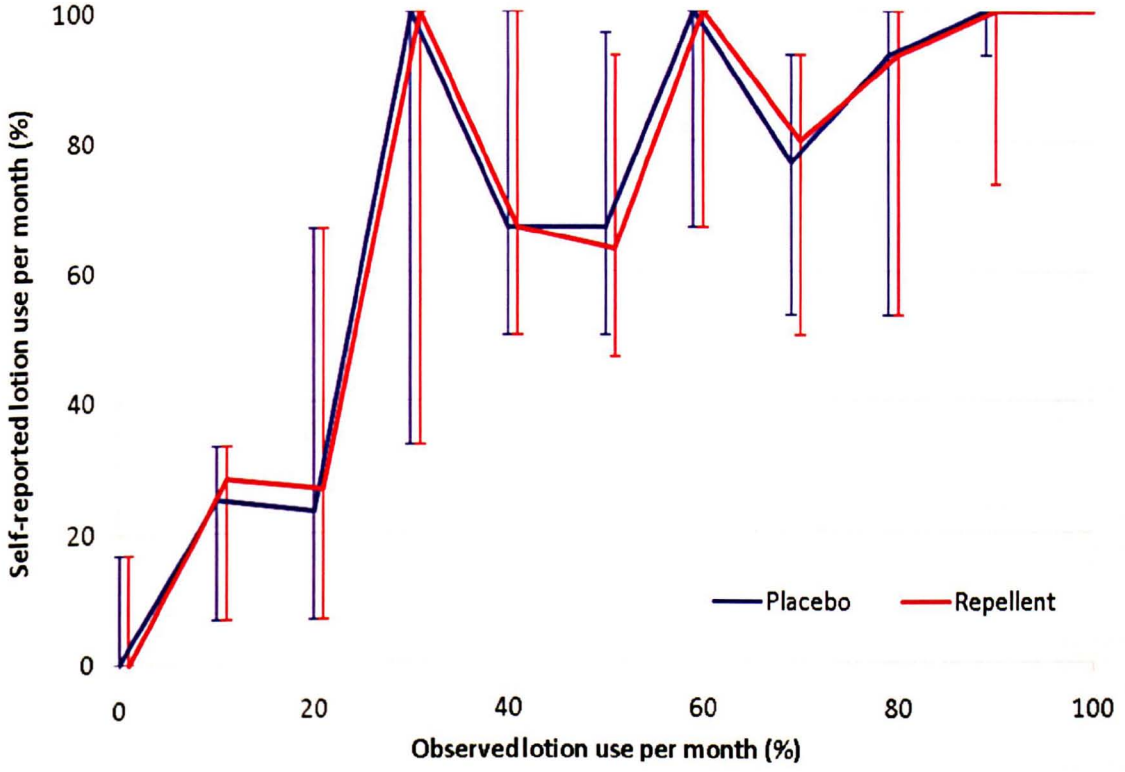


Figure 6.1. Median and IQR for self-reported lotion use with observed lotion use for repellent and placebo users.

The most common reason for non-compliance in both repellent and placebo users was forgetting to use the lotion (repellent users 68.8%, placebo users 69.1%, p=0.402). During FGDs

some participants said that they only used the repellent when they perceived mosquito biting to be bad, for example in the forest rather than the village. Although seasonal mosquito density for the study area is not available, malaria rates were highest between August and November when lotion use was highest (Figure 6.2). Other reasons for non-compliance included disliking the smell (repellent users 12.9%, placebo users 12.3%, p=0.212), and allergies which were slightly higher in repellent users (repellent users 3.8%, placebo users 3.2%, p=0.029). Fourteen percent of participants gave no reason for non-compliance.

Compliance with LLIN use was much higher than for lotion use regardless of treatment group (repellent users 97.0%, placebo users 97.3%, p=0.711). But a relationship was found between compliance with LLIN use and compliance with lotion use, and those participants who did not sleep under their LLIN every night were much less likely to use the lotion every day (χ^2=316.1, p<0.001).

Table 6.4 Problems reported about the repellent and placebo lotions non-compliers .

	Repellent users (%)	Placebo users (%)	Odds Ratio (95% C.I.)	P
Used the lotion every evening	61.29	62.21	0.96 (0.84-1.10)	0.559
Forgot to use the lotion	28.25	27.76	0.92 (0.76-1.12)	0.402
Did not use the lotion because of the smell	5.30	4.95	1.04 (0.85-1.27)	0.731
Did not use the lotion because of an allergic reaction	1.55	1.28	1.17 (0.86-1.59)	0.332
Did not use the lotion because it did not stop bites	0.28	0.43	0.63 (0.34-1.15)	0.131

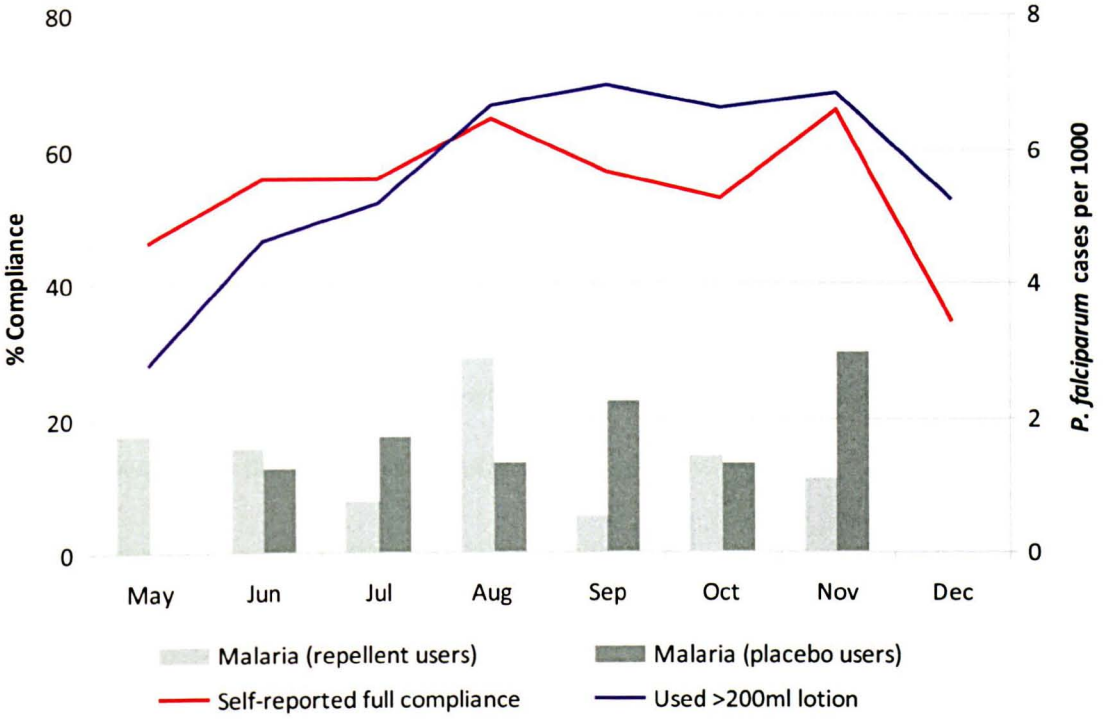


Figure 6.2 Percentage of participants who self-reported full compliance and those who used over 200ml per month, bars show *P. falciparum* rates in both treatment groups.

6.4.4. Perception of biting and methods of personal protection

Evening outdoor activity was revealed in the FGDs: people reported that at this time they could be walking from the rice fields or to the river, mending nets, eating, watching television or drinking and socialising. People would be exposed to outdoor biting mosquitoes during all of these activities as even those that take place in and around the house would either be in the open area underneath a stilted house, or on the veranda if the stilts were not high enough. But what also emerged in the FGDs was that people in this area are often active and outside their house at night. Men reported that they would start fishing in the river at around 11pm using lights to attract fish. During the peak of the wet season young children would also be collecting frogs as a supplement to their diet from flooded rice fields at around the same time. The ITT analysis found that men had almost twice the risk of *P. falciparum* infection compared to

women (men 1.8 cases per 1,000, in women 0.9 per 1,000, rate ratio: 0.54, 95% CI: 0.32-0.93, p=0.027). Visiting the forest is known to be a risk factor for malaria in Southeast Asia [183], and might explain the difference between infection rates between the genders. When asked whether the forest or the rice field had highest biting, 86% people chose the forest. Both men and women spent similar amounts of time away from the village during the day, but men were more likely to sleep away from the village (Table 6.4). FGDs suggested that while both men and women visited the forest, longer overnight trips were usually undertaken by groups of adult men whereas couples or family groups would only visit during the day and in general women who slept away from the village would be at their rice fields.

Table 6.5. Time spent away from the village by men and women.

	Men	Women	p
Mean days away from village per month	15.3	15.1	0.368
Spent >20 days away from village per month	35.0%	34.77%	0.705
Mean nights away per month	7.1	6.7	0.007
Spent >7 nights away from village per month	32.1%	29.9%	<0.001

Participants were asked whether they thought they had suffered malaria in the previous year regardless of RDT test results and if they knew how it had been transmitted. It should be noted here that the word for malaria in Lao language actually translates as 'mosquito fever' so the link to mosquitoes is clear. But with the high proportion of ethnic minorities in this trial it was desirable to see whether mosquito bites were still linked to malaria. Almost 40% of people believed that LLINs would protect from malaria, and this was unaffected if the person had previously had malaria (malaria 39.1%, no malaria 39.6%). Whereas the belief that repellents could protect from malaria was significantly lower amongst participants who thought they had caught malaria in the previous year (malaria 12.2%, no malaria 21.7%, p=0.014). Another

method of reducing insect biting described during the FGDs was to wear long clothing and even cover the face when visiting the forest or rice fields. Smoke, produced by a variety of methods, was used to drive away insects. Cooking fires kept mosquitoes away from the house or forest camps in the evening. Ropes can be made of tissue or rice leaves (from seedlings before transplantation) which are then burnt close to the body when travelling through the forest. One FGD participant even said that smoking cigarettes would reduce biting. Citronella was also mentioned during FGD, simply crushed and mixed with water and applied to the skin, although there were complaints that this was not effective for more than two hours. The trial repellent was generally thought to be more convenient than these methods.

6.5. Discussion

6.5.1. Compliance

The repellent was popular and acceptance was high although there was little history of topical repellent use in the area prior to the trial. There is a tradition of insect repellents, but as found our FGDs and by other researchers these are usually burnt as torches or incense rather than applied to the skin [81]. The compliance with repellent or placebo lotion use was around 60%, which compares well with other trials. Compliance of over 85% has recorded in Thailand but this trial used a product that was already well accepted by the user group and compliance of over 98% in a Bolivian trial was achieved by stringent nightly checks [131]. A much lower level of compliance, around 20%, was recorded in a trial of repellent soap where the intervention was on sale rather than provided free of charge [132]. In our study we found a weak but consistent relationship between self-reported and observed data for compliance. About 30% of participants presented inconsistent data, where they had reported using the repellent every evening in a month whilst actually using less than 200ml of lotion. These results suggest an adjustment of the inclusion criteria for the clinical trial analysis may be advisable, perhaps excluding participants with these inconsistent data.

Comparisons between self-reported and objectively measured data have been made in antiretroviral therapy studies where patients are required to take medication daily over the long-term and patient recall over several weeks or months make their findings relevant to this study [184-186]. Self-reporting always overestimates the compliance as there is social pressure to appear to comply with treatment. One study ranked patients by their likelihood to give a 'socially desirable response' and found this significantly improved predictions of clinical response to self-reported treatment [186]. Other trials have found consistent results, pre-operative physical activity in bariatric patients was identical when measured by self-reporting and objective measurements from an accelerometer, but self-reported post-operative physical activity was 11 times higher than that measured by the accelerometers [187]. There was great similarity in our study between self-reported full-compliance and full compliance as defined by use of at least 200ml of lotion. Comparison with similar trials suggests that this cut off may be too low and could be overestimating true compliance.

Although LLIN use was much higher than use of either lotion, the compliance with both interventions was closely related and the percentage of participants who were fully compliant with lotion use was twice as high amongst participants who were also fully compliant with LLIN use as those who were not. The trial was carried out in an area of low malaria transmission, so people may have chosen not to use an LLIN or repellent because they believed they were not at risk of malaria. There is some indication that this could be the case from raw malaria figures (LLIN compliant 1.3 *P. falciparum* cases per 1000, LLIN non-compliant 0.7 cases per 1000), but a more in depth study of village level malaria and compliance would make this clearer.

6.5.2. Social acceptability of repellent

The smell of the repellent was found more offensive than the placebo lotion, suggesting that the smell of DEET itself was what participants objected to, although this did not translate to a reduction in compliance. However it may be that higher concentrations would be less acceptable, particularly for long term use. Allergies were reported by about 16% of

participants and there was no difference between repellent and placebo groups, meaning DEET was not responsible for all adverse reactions. This was high when compared to the proportion who gave an allergy as the reason for non-compliance, less than 4% in both groups. FGDs suggested there may have been some confusion over the definition of an allergic reaction. Allergies were defined with local health staff prior to the trial as a rash, redness or swelling of the skin where lotion was applied or breathing difficulties. But FGD participants reported 'allergic reactions' when the lotions were misused such as applying to wounds or accidently getting into the eyes. This kind of accidental exposure to wounds might be commonly experienced by participants carrying out agricultural work, but as it was recognised that the misuse rather than the lotion was to blame this had little effect on overall compliance. Safety directions were given at the start of the trial during demonstration of lotion use and enforced by posters in villages and by illustrated directions on the bottle labels. However, as participants were reporting adverse effects from misuse and over-use it was clear that participants were not always following these instructions. Not all over use resulted in adverse reactions and observations from FGDs found women using lotions for cosmetic rather than insect repellent reasons, this is echoed in other trials where repellent use was found to be highest amongst women and unrelated to malaria knowledge [188]. LLIN use is promoted in southern Lao PDR by interactive educational shows, and it may be necessary to use this type of communication to promote appropriate use of repellent.

6.5.3 Malaria exposure

Across Southeast Asia young men are usually found to be at greater risk of malaria and this is often explained by men making more visits to the forest and thereby coming into greater contact with *An. dirus,* the major malaria vector of the region [20-22, 155]. However in the Lao PDR village trials have mostly found no difference between male and female infection rates [45-47]. This discrepancy could have been explained by the exclusion of forestry workers from these village-based surveys, these are often young male immigrants and are at high risk both

through exposure and because they may have come from malaria-free areas and have no natural immunity. However, our trial also excluded this group but still found malaria rates more in line with the rest of Southeast Asia with men at greater risk of infection. Differences in study methodology are most likely to explain the differences. Local people spend much of the rainy season away from their villages living nearer to their rice fields. Participants in the repellent trial were recruited before the wet season and asked to return to the village once a month for follow-up surveys throughout the rains. Other trials have recruited participants present in the village when researchers turned up, which could have excluded those living away who are potentially at greater risk. Control programmes in the Lao PDR need therefore to focus more on this behaviour for example by supplying single man LLINs that can be taken into the forest.

The intervention trial used repellent in the evening and LLINs at night, but evidence from FGD suggests that the repellent would be useful at night as well. Men and children were outdoors at night carrying out activities such as fishing or catching frogs. This night time exposure is likely to be an important risk factor for malaria and consideration of this is recommended in future control programmes.

6.5.4. Conclusion

The trial repellent and placebo lotion were generally well liked and use was high, but improvements could be made to make them more accepted. Spray formulations or repellent wipes that could be easily transported or a water resistant formulation that could protect during fishing might be well appreciated. Overall, topical repellents were successfully introduced in the study area despite the lack of their traditional use. Control programmes that include repellents would need to address education, particularly focusing on appropriate and safe use, to maximise their uptake.

Chapter 7: A meta-analysis of randomised controlled trials of topical insect repellent to reduce malaria.

7.1 Abstract

Background: Malaria is major cause of mortality and morbidity in developing countries. The vector control strategies currently recommended by the World Health Organization (WHO) are long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Both these strategies target mosquitoes that rest and feed indoors. But in Southeast Asia and South America local vectors are primarily outdoor biting. Therefore there is a real need for tools such as insect repellents that can prevent outdoor biting.

Objectives: To assess the impact of topical insect repellents on malaria cases.

Methods: Published and unpublished trials were sought using CAB Abstracts, Cochrane library, EthoS, LILACS, Open Grey, Pubmed, Web of Science, the WHO Library, ClinicalTrials.gov and Current Controlled Trials. Reference lists of papers were checked and researchers contacted for unpublished data. The search criteria were cluster and randomised trials of topical insect repellent compared to placebo or no treatment to reduce malaria. Trials were assessed for inclusion, checked for bias and data extracted and analysed.

Main results: Four trials including the Lao PDR repellent trial met the inclusion criteria. The average protection from *Plasmodium falciparum* malaria by the use of topical insect repellents was 30% although this was not significant (rate ratio: 0.70, 95% Confidence interval: 0.42-1.16, p=0.16). Similarly the 38% average protection against *P. vivax* infections did not reach significance (rate ratio: 0.62, 95% CI 0.18-2.16, p=0.45).

Conclusions: In comparison with other repellent trials, the results from the Lao PDR showed generally lower protection from repellent use. However the effect was within the scope of other trial results and in fact only one trial showed a significant decrease in *P. falciparum*

infections. The variation between trials was very high, which could be explained by different background malaria rates, different compliance levels or variation between intervention formulations. There was also suggestion of publication bias that excluded trials showing low effect from repellent. Overall the results show that topical insect repellent is not a suitable wide-scale intervention against malaria.

7.2 Background

The World Health Organization reported 655,000 confirmed deaths from malaria in 2010 [1], but the actual figure could be about twice as high, with a recent study estimating around 1.2 million deaths [2]. The Roll Back Malaria initiative [189] recommends attacking malaria on three fronts: (1) vector control through long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), (2) preventative therapy for high risk populations and (3) accurate diagnosis with appropriate treatment. LLINs reduce malaria mortality and morbidity by approximately 50% in a range of settings [67]. However, LLINs and IRS both work best against indoor biting mosquitoes. The most important malaria vectors in the Greater Mekong Subregion (GMS) are *Anopheles dirus*, *An. minimus* and *An. maculatus* which often bite in the early evening and outdoors before people can be protected by LLINs [51, 56]. There is also evidence that the indoor biting African vector *An. gambiae* s.s. is being replaced by the outdoor biting vector *An. arabiensis* [136, 190, 191]. This change has been linked to the use of indoor insecticides in LLINs and IRS, but whatever the cause the effect is the same, increasing the need for new tools to protect people against outdoor biting vectors.

It is likely that people have been using repellents to prevent insect bites since pre-history [79]. Early repellents were largely plant derived and include some still in use today such as citronella (oil derived from plants of the *Cymbopogon* genus), neem (leaves from *Azidarachta indica*) and lemon eucalyptus (*Eucalyptus maculata citriodon*). Synthetic insecticides were first developed during the twentieth century, and *N,N*-diethyl-m-toluamide (DEET) the most effective repellent to date was developed in the 1950s [77]. Topical insect repellents are very successful at

reducing outdoor biting at any time of the day from a wide range of insects, but this protection is short-lived. The current 'gold standard' repellent, DEET, applied topically will provide up to six hours of protection under field conditions [125, 127]. This short period of effect means topical insect repellents are not suitable as a sole intervention, but they might be an effective complementary tool to LLINs.

7.3 Objectives

To assess the impact of topical insect repellent on *Plasmodium falciparum* and *P. vivax* cases. The hypothesis is that insect repellent used in areas of outdoor biting will provide additional protection above that provided by LLINs alone.

7.4 Methods

7.4.1 Literature search and study selection

A literature search was performed to find randomised controlled trials of insect repellents used to prevent malaria. The search terms "insect repellent" and "malaria" were entered into more focused databases: CAB Abstracts, Cochrane library, EthoS and LILACS. Grey literature was also sought using the same search terms in the Open Grey database. Additional terms "trial" or "randomised controlled trial" were used to narrow the results from more general databases: Pubmed, Web of Science and the WHO Library. Unpublished studies were searched for in clinical trials databases ClinicalTrials.gov and Current Controlled Trials and results requested from the authors.

The results were checked for duplicates and the resulting references screened for inclusion in the qualitative and quantitative analyses. Trial interventions included any topical insect repellent regardless of active ingredient or concentration used. Repellent impregnated clothing was excluded as this would involve a different level of behaviour change from users. Space repellents were also excluded as the effect would be difficult to directly compare to topical applications. Outcomes reported had to include parasite rates of *P. falciparum* or *P.*

vivax malaria measured by blood smears or rapid diagnostic tests. Trials involving travellers from developed countries were also excluded as the compliance and trial duration would be very different to trials involving local populations. Trials had to be randomised controlled trials to be included in the quantitative analysis, but other studies such as case-control studies were also included for discussion in the qualitative analysis.

7.4.2 Data collection and analysis

Standard forms were used to collate trial location; study population; randomisation; blinding methods; repellent formulation; estimated coverage or compliance and method of estimation; type of control; co-interventions; outcome measures and length of follow-up from each trial. If not presented in the report, the trial location was used to infer malaria endemicity, *Plasmodium* species and *Anopheles* vectors present.

Review manager 5.1 was used to calculate rate ratio, rate difference, summary rate ratio and summary mean difference. If the model I^2 value was >50% indicating significant heterogeneity between studies random effects were used to calculate confidence intervals.

7.4.3 Risk of bias

Randomised controlled trials were assessed for risk of bias through recruitment bias, generation of allocation sequences, allocation concealment, blinding, baseline imbalance, missing data and selective reporting. Recruitment bias would arise when recruitment procedures exclude groups of participants, a common example would be the exclusion of certain age groups, which means that the findings of the trials may not apply to these excluded groups. Trials were given an assessment of 'A' meaning the recruitment process was fair and representative, 'B' indicating the recruitment process was unclear or not described, and 'C' if the recruitment process was clearly biased. The generation of the allocation sequence describes the method of randomisation. Good randomisation ensures the treatment groups are comparable and each individual or cluster should have an equal chance of ending up in each group. Trials were assessed as 'A' acceptable randomisation method, 'B' if trial described

as randomised but method not described or 'C' poor randomisation. Allocation concealment and blinding are closely related but slightly different. In a blinded trial the participants do not know which treatment or placebo they have been given, in a double-blinded trial the people assigning the intervention also do not know the identities of the treatment. Allocation concealment means that steps have been taken to ensure nobody concerned with the data collection or analysis know or can find out which treatment they have been given, so usually the key to identifying the treatments is kept securely until the all data analysis had been completed. Therefore trials were rated 'A' if the were double-blinded and had taken step to conceal the allocation sequence from investigators, 'B' if stated as blinded but not clear who was blinded and allocation concealment not fully described or 'C' if the blinding or allocation concealment were not acceptable. Imbalances in baseline measurements show that the randomisation may not have worked well, and that there are significant differences between the two groups. Trials were rated 'A' if treatment groups were similar across a range of measurements, 'B' if treatment groups are similar only for baseline malaria rates or 'C' if treatment groups showed significant differences at baseline. Trials with a high level of missing data that was likely to be related to the outcome or was unbalanced between treatment groups were given a 'C' assessment, if missing data is not reported clearly a 'B' and low missing data or balanced missing data between groups an 'A'. Trials were also checked for selective reporting, so a trial reporting all pre-specified outcomes was assessed as 'A', if outcomes were not clearly pre-specified 'B' or not all pre-specified outcomes were reported a 'C'.

7.5 Results

7.5.1. Study selection

Fifty-six references were identified from the literature search, but 48 were excluded from both quantitative and qualitative analyses because they concerned the wrong intervention (10), wrong outcome (16), wrong study population (1) or wrong trial type (13) or a combination (8).

7.5.2 Study characteristics

Eight studies of insect repellent against malaria were identified, including seven randomised control trials which were carried out in Bolivia, Ecuador and Peru, India, Lao PDR, Pakistan, Tanzania and Thailand and a case-control study in Afghanistan. The study characteristics of these trials are summarized in Table 7.1. Four of these studies were excluded from quantitative analysis. Two, the trials in Ecuador and Peru, and Thailand could not be entered into the meta-analysis because they did not report full data on cases and person time at risk [128, 129]. The Indian trial also failed to report separate incidence rates for *P. falciparum* and *P. vivax* [192]. The case-control study was could not be included in the meta-analysis, however it was included in the qualitative analysis as it was a well-run study giving informative results [132].

Table 7.1. Study characteristics of randomised controlled trials of insect repellent against malaria.

Location	Trial characteristics
Afghanistan [132]	**Trial type:** Case-control study **Study population**: Fever patients from two clinics in eastern Afghanistan. Cases were confirmed by microscopy. **Repellent:** Repellent soap containing 20% DEET and 0.5% permethrin was promoted by ministry of health staff in the year prior to the study. **Other behaviours recorded:** Age, gender, insecticide treated net use.
Bolivia [131]	**Study population**: 860 households recruited from rural and peri-urban communities. Up to 20% of households per village were recruited. Participants were aged >10 years and 45.2% were female. **Randomisation:** Sequential alternate system used to randomise households. **Blinding**: Field staff and study participants were blinded to allocation. **Intervention:** 30% PMD lotion.

	Coverage: Compliance measured by questionnaires, observed volume of lotion used and random sniff checks. 98.5% of participants used lotions >90% of the time. **Control:** 0.1% clove oil **Co-intervention:** Treated nets **Outcome**: *P. falciparum* incidence as measured by active monthly detection by RDT and *P. vivax* incidence passively detected by blood slide at a local clinic. **Length of follow-up:** 4 months
Ecuador and Peru [128]	**Study population:** 18 rural communities on the north coasts of Ecuador and Peru. **Randomisation:** Simple randomisation of matched pairs. **Blinding:** None **Intervention:** Repellent soap containing 20% DEET and 0.5% permethrin **Coverage:** 50-70% when soap was distributed free, 6% when soap was sold. **Control:** Untreated **Co-intervention:** None **Outcome:** Self-reported malaria episodes. **Length of follow-up:** 7 months
India [192]	**Study population:** 2 rural villages. **Randomisation:** Not described **Blinding:** None **Intervention:** Insect repellent, proprietary name Enteemosq, active ingredient not given. **Coverage:** Not estimated **Control:** Untreated

	Co-intervention: None, but chemotherapy guidelines changed halfway through the trial possibly triggering large decreases in malaria incidence. **Outcome:** Malaria cases diagnosed by microscopy **Length of follow-up:** Two years
Lao PDR	**Study population**: 1,597 households recruited from agricultural communities. Up to 25% of households per village were recruited. Participants were aged 6-60 years and 54.8% were female. **Randomisation:** Equal groups allocation of households stratified by village. **Blinding**: Data analyst, trial manager, field staff and study participants were blinded to allocation. **Intervention:** 15% DEET lotion **Coverage:** Compliance measured by self-reporting, observed volume of lotion used and random infrequent sniff checks. 58% of participants used lotions >90% of the time. **Control:** Placebo lotion **Co-interventions:** LLINs **Outcome:** Active detection of *P. falciparum* and *P. vivax* cases by monthly RDTs **Length of follow-up:** 5-8 months (average 6.3)
Pakistan [130]	**Study population**: 127 households recruited from a refugee camp on Afghan border. 25% of households in camp were enrolled. Participants were aged >5 years and 49.2% were female. **Randomisation:** Simple random allocation of households. **Blinding**: Study participants were blinded to allocation. **Intervention:** 20% DEET and 0.5% permethrin soap **Coverage:** Twenty (16%) households interviewed at end of study, 19 (95%)

	reported using the repellent 'regularly'. **Control:** placebo lotion **Co-interventions:** None **Outcome:** Passive detection of *P. falciparum* and *P. vivax* cases diagnosed by microscopy. **Length of follow-up:** 5 months
Tanzania [178]	**Study population**: 937 households recruited from a rural village. 50% of households in a village were recruited. Participants were aged >6 months and 55.3% of household heads were female. **Randomisation:** Sequential alternate allocation of clusters of 47 households. **Blinding**: Not described. **Intervention:** 15% DEET lotion **Coverage:** Compliance measured by self-reporting and compliance with repellent reported at 89% and placebo 68% **Control:** Placebo lotion **Co-intervention:** LLINs **Outcome:** Passive detection of malaria cases confirmed by RDT at local dispensary. **Length of follow-up:** 14 months
Thailand [129]	**Study population:** 897 women 3-7 months pregnant recruited from Karen refugee camps in western Thailand. **Randomisation:** Method not described **Blinding:** Method not described **Intervention:** Repellent lotion containing 20% DEET and thanaka (*Limonia acidissima*) **Coverage:** Compliance self-reported at 90.5% and actively detected at 84.6%

Control: A placebo formulation containing thanaka

Co-interventions: None

Outcome: Active case detection by weekly blood smears

Length of follow-up: 18 weeks (median)

7.5.3 Outcomes of individual studies

The outcomes of the four studies excluded from the meta-analysis are described here, and Tables 7.2 and 7.3 show reported outcomes from included trials. In India, no effect was found on malaria rates following distribution of repellent for two years [192]. Again no significant reduction was found in malaria rates in Ecuador and Peru where repellent soap (20% DEET and 0.5% permethrin) was distributed or sold [128]. In Thailand reductions in malaria rates were recorded in repellent users, but the lower than expected overall malaria rates meant that samples were to low for these reduction to reach significance [129]. A case-control study in Afghanistan found that the use of repellent soap reduced the odds of malaria by 45% even after accounting for confounders such as LLIN use [132].

Table 7.2. Outcomes (cases/person time at risk) of randomised controlled trials of repellent against *P. falciparum* and resulting rate ratios with 95% CI between trial arms.

Study	Repellent	Control	Rate Ratio (95% CI)
Bolivia [131]	1/7,706	6/7,468	0.16 (0.02-1.34)
Lao PDR	29/22,145	29/21,879	0.88 (0.45-1.71)
Pakistan [130]	23/618	47/530	0.42 (0.26-0.68)
Tanzania [178]	122/2,586.25	138/2,549.12	0.87 (0.69-1.10)

Table 7.3. Outcomes (cases/person time at risk) of randomised controlled trials of repellent against *P. vivax* and resulting rate ratios with 95% CI between trial arms.

Study	Repellent	Control	Rate Ratio (95% CI)
Bolivia [131]	14/7,673	66/7,336	0.20 (0.11-0.36)
Lao PDR	13/22,145	14/21,879	0.96 (0.54-1.71)
Pakistan [130]	103/618	62/530	1.42 (1.06-1.91)

7.5.4. Meta-analysis

The combined summary rate ratio for *P. falciparum* infection rates was 0.67 (95% CI 0.42-1.09, p=0.11), giving a protective effect of 30% from repellent use although this did not reach statistical significance (Figure 7.1). The overall effect on *P. vivax* infection rates was similar to that for *P. falciparum* but was again not statistically significant (rate ratio: 0.65, 95% CI 0.18-2.34, p=0.51). Heterogeneity was very high (I^2=70% in the *P. falciparum* analysis and I^2=94% in the *P. vivax* analysis) so random effects were used to calculate confidence intervals for the overall effect.

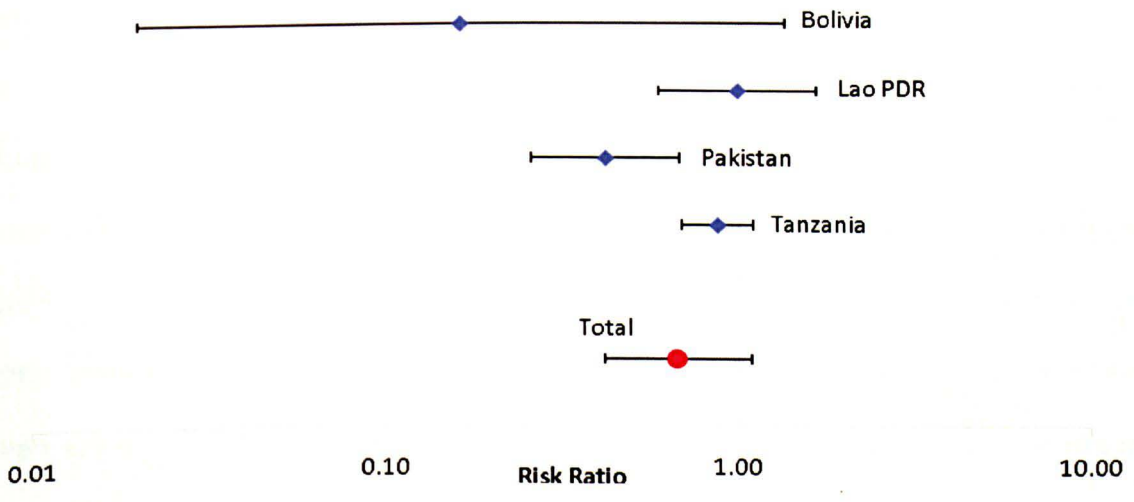


Figure 7.1. Rate ratios and 95% confidence intervals from randomised controlled trials of insect repellent against *P. falciparum* malaria.

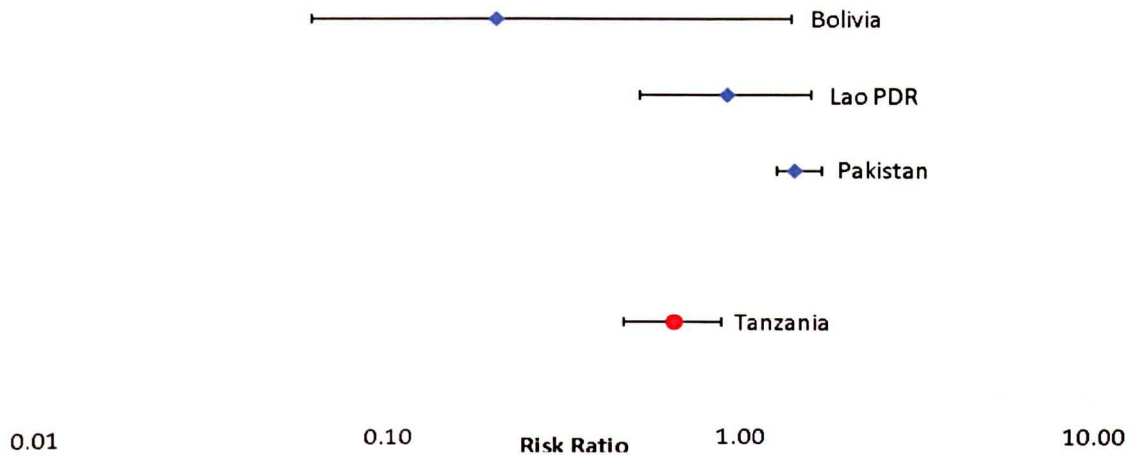


Figure 7.2. Rate ratios and 95% confidence intervals for from randomised controlled trials of insect repellent against *P. vivax*.

7.5.5 Risk of bias

An overall comparison of the risk of bias in each trial is shown in Table 7.4. Recruitment bias was low in three of the four studies, in the Lao PDR study, the use of village elders to choose trial households may preferentially have selected individuals whose compliance with the intervention and malaria monitoring was higher than in general. All trials used a fair system to generate allocation sequences, either simple randomisation or lottery systems. Bias from a lack of allocation concealment was less easy to assess. In Pakistan the placebo was a lotion which was compared to a repellent soap formulation, meaning that field workers may have known the treatment allocations throughout the trial. The other three trials used similar placebo and intervention products, but only the Lao PDR trial reported that the treatment key was kept away from researchers. Baseline imbalance was generally low, with some minor differences reported in the Bolivian and Tanzanian trials. Loss to follow-up and missing data were low in the Bolivian and Tanzanian trials, however the loss to follow-up was over 10% in the Lao PDR and this information was not reported from Pakistan. All trials reported the pre-determined outcomes of *P. falciparum* and *P. vivax* cases. Blinding was attempted in all trials, but with repellents, the possibility always remains that participants using the placebo quickly

notice that they continue to be bitten. This problem would particularly apply to trials where there were only two treatment codes for repellent and placebo, and participants were able to compare the efficacy of the two products. The Tanzanian trial may have reduced the likelihood of this comparison by randomising groups of geographically close households to the same treatment. All trials provided results of statistical analysis that took the effect of clustering into account.

Table 7.4. Methodological bias in trials included in quantitative analysis.

Potential source of bias	Bolivia [131]	Lao PDR	Pakistan [130]	Tanzania [178]
Recruitment	A	B	A	A
Generation of allocation sequences	A	A	A	A
Allocation concealment	B	A	B	B
Baseline imbalance	A	A	A	A
Missing data	A	B	B	A
Selective reporting	A	A	A	A
Blinding	B	B	B	A
Analysis allows for clustering	A	A	A	A

7.6 Discussion

7.6.1 Summary of evidence

The combined effect of repellent use from four randomised controlled trials was a 33% reduction in *P. falciparum* and a 35% reduction in *P. vivax*, although these differences were not statistically significant. There have only been four fully reported trials and any attempt to compare the results is confounded by varying background rates of malaria, user compliance and co-interventions.

7.6.2 Limitations

Heterogeneity was very high in the meta-analysis indicating substantial variance between the studies. As there were so few studies, it was not thought appropriate to create subgroups to account for some of the important differences between studies that could contribute to this high heterogeneity. The most obvious difference is in study location which would lead to varying background malaria rates. Malaria rates in the control groups ranged from 0.1-11.7 cases per person per year. and mathematical modelling suggests that lower background malaria rates mean the repellent intervention could have a greater effect [137]. The interventions also varied; DEET, permethrin and PMD were all used at different concentrations and formulations. The formulation will have a large impact on dosage applied, so it is difficult even to make a judgement on a 15% DEET lotion being a less efficacious repellent than a 20% DEET soap. Compliance varied greatly between studies from 98% in Bolivia to 58% in the Lao PDR. Compliance is very difficult to assess in large trials as direct observation is only practicable in a small number of participants. Most of the trials used a combination of self-reported data confirmed by a small number of direct observations. The mathematical model mentioned above suggests that compliance is the most influential component on a repellent's potential to reduce malaria. All of these factors made combining the studies to create an

overall figure for protection difficult, although with more studies it may have been possible to take factors such as malaria endemicity and compliance into account.

It was also evident that publication bias may have affected the meta-analysis. Two trials were excluded from the meta-analysis because full data could not be found in the literature for case numbers. These were trials that did not find any effect and their publications may have been compressed because of this rather than any lack of methodological quality. Therefore despite not finding any significant value for repellent protection it is likely that the overall protection has still been overestimated.

Despite vector control having a major role in current malaria control and elimination policies, very few meta-analyses have yet been carried out on vector control interventions. Two Cochrane reviews have been produced examining the effectiveness of LLINs at reducing malaria, one of which concentrates on malaria in pregnancy. The general review found only fourteen studies to include in analysis [67]. A single meta-analysis has been produced on IRS, which included six studies and found that there was too little high quality data to establish an overall effect [193]. Mosquito larval source management and mosquito control using larvivorous fish are both awaiting a full meta-analysis, although a protocols have been published for both [194, 195]. One other Cochrane review has been published on electronic mosquito repellents which found no protective effect [87]. The scarcity of these meta-analyses and the small number of studies that are included illustrate how urgent the need is for further well-designed trials of vector control interventions.

7.6.3 Conclusions

The randomised controlled trial of 15% DEET against malaria in southern Lao PDR found no evidence of an effect on malaria. When put in the context of the results of other repellent trials, the measured effect is low but not significantly different from other outcomes, and much lower than the 50% effect that the trial in the Lao PDR hoped to detect.

Although LLINs do work well, outdoor biting in South-east Asia means they are unlikely to be sufficient as a sole intervention method in this region. Signs of changes in mosquito behaviour in Africa also suggest protection against outdoor biting might be needed in other areas too. Repellents can protect against outdoor biting, but further trials of topical repellent aimed at developing a wide-scale malaria control tool are not recommended based on these results. What is needed now are trials of alternative modes of repellents delivery such as long-lasting repellent impregnated into clothing as well as trials that can measure the impact of repellents on other vector-borne diseases.

Chapter 8: Assessing the efficacy of topical insect repellents to reduce malaria in southern Lao PDR.

8.1. Abstract

Malaria remains a serious threat in the Greater Mekong Sub-region (GMS), not just from the direct impact on human health, but also from the emergence and spread of resistance to artemisinin, the last remaining effective antimalarial. Therefore malaria control in this region is a high priority on a global as well as local scale. In southern Lao PDR, as across much of the GMS, malaria vectors bite outdoors in the early evening before people are protected by their bed nets. Lotions containing 10-20% *N,N*-diethyl-*m*-toluamide (DEET) were shown to protect users from 96-99% of biting by mosquitoes between 17.00 and 22.00h in a village in southern Lao PDR. 15% DEET was found to be the best choice of intervention as it provided the most reliable protection at the lowest dose. A randomised controlled trial was designed to test the effect of 15% DEET against malaria. A total of 1,597 households were recruited and randomised to either a 15% DEET or a placebo lotion. All households were also provided with long-lasting insecticide treated nets (LLINs). Intention to treat analysis found no difference between treatment arms after accounting for gender, socio-economic status and observed lotion use (rate ratio 0.96, 95% CI 0.54-1.71, p=0.886). According to protocol (ATP) analyses of participants who used the lotions over 90% of the time also found no effect from repellent use after other factors had been taken into account (rate ratio 1.45, 95% CI 0.53-3.99, p=0.467). The most important predictor of malaria incidence was a socio-economic score based on a combination of factors including housing materials, access to services such as electricity and ownership of key possessions like motorbikes. Lower wealth resulted in higher risk of malaria in both ITT and ATP models for outcomes of *P. falciparum*, *P. vivax* and overall malaria. While the repellent was well received and over 90% of participants said they liked using both the lotions, compliance was still low with fewer than 60% of participants using the lotions more

than 90% of the time. However, the results of the ATP analyses suggest that low compliance was not responsible for the lack of observed effect from the trial. The size of the effect on *P. falciparum* was lower than in other randomised controlled trials of repellent carried out in Bolivia, Pakistan and Tanzania. A combined rate ratio from all four trials found that repellent use was associated with a 33% reduction in *P. falciparum* incidence and a 35% reduction in *P. vivax* incidence. Two trials were excluded from this analysis as full data were not reported; both had non-significant results.

The outcome of this trial shows that topical insect repellent is not a suitable wide-scale intervention against malaria and does not provide significant protection over and above LLINs in an area of outdoor biting. However, repellents do undoubtedly reduce biting and therefore their potential to be effective intervention tools remains. Smaller-scale interventions could be more effective, such as targeting high-risk groups such as children, pregnant women or non-immune migrants. Alternatively repellent impregnated clothing could provide outdoor personal protection which requires much less of a behaviour change from the user than topical formulations.

8.2. Background

Malaria is a major health problem across the tropics, but the nature of the challenge varies between different regions. Malaria in Africa accounted for 78% of the 225 million cases and 91% of the 780,000 deaths caused by the parasites in 2009 [48]. In the Greater Mekong Sub-region (GMS) the major concern is the development of antimalarial resistance as genetic studies suggest this region is where strains resistant to chloroquine and pyrimethamine emerged previously, before spreading to other endemic areas [5, 196]. At present artesunates are the only remaining effective antimalarials and the first cases of artesunate-resistance have already been detected on the Thai-Cambodian border [197].

The current best method of malaria prevention is the use of long-lasting insecticidal nets (LLINs), but these have a major limitation in the GMS. The most important malaria vectors in the GMS are *Anopheles dirus*, *An. minimus* and *An. maculatus*, which bite in the early evening and outdoors before people are protected by their bed nets [51, 56]. Outdoor biting behaviour also makes control methods such as indoor residual spraying or house screening less effective. Larval control is limited as larval habitats are normally small water bodies found in the forest or forest fringe, challenging to access and identify [52, 55]. There is also evidence that vector behaviour in Africa is shifting towards outdoor and evening biting, perhaps in response to indoor insecticide use such as LLINs and indoor residual spraying [136, 190, 191], increasing the need for tools to protect against outdoor biting. Topical insect repellents seem to answer these issues with LLINs. They can protect against outdoor biting as easily as indoors, and are suitable for use at any time of the day.

One of the most obvious limitations with insect repellents is the duration of protection. The current 'gold standard' repellent, *N,N*-diethyl-m-toluamide (DEET), applied topically will provide up to six hours of protection under field conditions [125, 127]. In comparison, LLINs last up to five years and can be permanently suspended over the bed space further reducing the effort required by the user. This also means that topical repellents cannot provide all-night protection whilst people are asleep as this would require the user to wake and reapply during the night. The solution to this would be a more permanent repellent formulation, such as impregnated clothing.

Repellents can have an insecticidal effect in the laboratory, but under normal field conditions the repellent prevents direct contact and any lethal effects. LLINs have the advantage here too, as the insecticide reduces the mosquito population in the local area, reducing disease transmission for both net-users and non-users. Repellent users may actually increase the risk to nearby non-users by diverting biting pressure [121]. However, these disadvantages of repellents and advantages of LLINs do not necessarily apply to the outdoor biting scenario in

the GMS. The community effect of LLINs is likely to be diluted as fewer mosquitoes enter the house and come into contact with the insecticide. If the LLIN is carried into the forest for the night, killing a small number of mosquitoes here will likely have no impact on human health at all. Diversion of biting away from repellent users might not increase disease risk for other people as many of the GMS mosquito vectors will readily bite animals as well as humans [56].

Therefore while repellents are not suitable as a sole control method, when used in conjunction with LLINs they have the potential to provide more complete protection in areas of outdoor biting. A randomised controlled trial was therefore designed to test the hypothesis that repellents could provide additional protection against malaria when used alongside LLINs in southern Lao PDR.

8.3. Entomology Surveys

Entomological surveys were carried out in Gueng Makheua, Saysettha District, Attapeu Province as a typical lowland village, surrounded by a mix of open woodland and rice fields. The village was approximately 30km from the district hospital, but was the site of a local health centre servicing four villages. Routine entomological surveys using light traps and larval sampling were carried out in July 2009 to gather background information on the species found in the study area. In July 2010 human landing catches with 10%, 15% and 20% DEET and a control lotion were carried out over sixteen evenings between 17.00h and 22.00h in a Latin-square rotation to compare the efficacy of these concentrations.

The most common mosquito species in both landing and light trap collections included members of the *Culex vishnui* subgroup known to be vectors of Japanese Encephalitis (JE) as well as members of the *Stegomyia* and *Armigeres* genera which feed early in the evening. On average light traps caught 50 mosquitoes per trap-night and all-night landing collections 15 mosquitoes per person-night. About three times as many species were also recorded from the light traps, but these would represent mosquitoes attracted indoors which may only be to rest

rather than feed on humans sleeping indoors. Therefore in a region with such high mosquito species diversity such as Southeast Asia, landing catches are necessary to establish the species that are actually biting humans and therefore of potential importance for disease transmission.

All DEET concentrations tested gave significant protection from biting compared to the placebo over the five hours of testing. After controlling for night and collector variation, 10% DEET gave 96.1% protection (95% C.I. 92.4-99.0%), 15% DEET provided 98.9% protection (95% C.I. 96.0-100%) and 20% DEET gave 98.1% protection (95% C.I. 95.0-100%). The greater variation found in the protection from 10% DEET means that 15% would be a safer recommendation for use in the repellent trial, where environmental conditions will be much more variable and repellent use will be mostly unsupervised.

8.4 Randomised controlled trial of topical repellent against malaria

A double blind, household randomised, placebo controlled trial of insect repellent to reduce malaria was carried out in southern Lao PDR to determine whether the use of repellent and LLINs could reduce malaria more than LLINs alone. Three hundred household were recruited in June 2009 and a further 1,297 in April 2010 giving a total of 1,597 households which included 7,979 participants. In total 795 households (3,972 participants) were assigned to use a 15% DEET lotion and 802 households (4,007 participants) a placebo lotion. Randomisation was stratified by village and participants, field staff and data analysts were all blinded to the group assignment. All households received new long lasting insecticidal nets (LLINs) the current best practice. Participants were asked to apply their lotion to exposed skin every evening from 18.00h until retiring, adults were provided with 300ml per month and children under 12 years with 200ml. *Plasmodium falciparum* and *P. vivax* cases were actively identified by monthly rapid diagnostic tests. Five rounds of follow-ups were completed in 2009 and eight in 2010.

An initial intention to treat (ITT) analysis was performed on all data. Household socio-economic scores, age, gender, nights slept under an LLIN, nights spent away from the village, self-reported lotion use and observed volume of lotion used were tested using non-parametric univariate tests for inclusion in the model. Those with a significant association with the outcome at $p<0.2$ were then considered for inclusion in a multi-level mixed effects Poisson regression model adjusted for intra-cluster variation by random effects methods. Variables maintaining their association in the multivariable model at $p<0.05$ were kept in the final model. According to protocol (ATP) analyses were then performed which excluded participants who complied with LLIN and lotion use less than 90%, 75% and 50% of the month. The effect of LLIN use on malaria was estimated by comparison of malaria rates before and after baseline.

Intention to treat analysis included 1,398 households followed up for a maximum of eight months. No effect from the use of repellent was found on malaria incidence (IRR: 0.96, 95% CI: 0.54-1.71, p=0.886). A higher household socio-economic score indicating higher wealth was found to decrease the risk of malaria. Women were found to have approximately half the risk of *P. falciparum* malaria compared to men (IRR: 0.54, 95% CI: 0.31-0.92, p=0.025), although gender was not significant in *P. vivax* models. ATP analysis produced consistent results, no effect from the use of repellent was found, while lower wealth increased malaria risk.

8.5 Acceptance of topical repellents as an intervention tool

Compliance with LLIN use and lotion use were first compared separately between treatment arms and then a chi-squared analysis of the relationship between compliance with each intervention was performed. Focus group discussions were held to establish the opinion of the repellent users to the lotions used and explore reasons for non-compliance. Questionnaires administered throughout the trial collected similar information. Reports of adverse events were compared between treatment groups using a chi-squared test. A logistic regression was run to examine treatment group, age and gender as predictors for the reports of allergic

reactions. A comparison between self-reported lotion use and the amount of lotion returned to health staff was also made.

Almost two-thirds of participants reported that they had used the lotion at least 90% of the time (repellent users 65.5%, placebo users 66.2%, χ^2=2.47, p=0.116). However less than half of participants used more than 90% of the lotion supplied each month (repellent users 47.4%, placebo users 48.1%, χ^2=2.31, p=0.129). There was a weak but consistent relationship found between self-reported lotion use and the amount of lotion that was observed to have been used (pair-wise correlation r^2=0.19) and 61.9% of paired observations were within 10% of each other. The most common reason for not using the lotions every night was forgetfulness, with no difference between treatment groups (repellent users 68.8%, placebo users 69.1%, χ^2=0.18, p=0.675). Only 20.2% of placebo users complained that the lotion was failing to reduce biting, although this was significantly higher than repellent users (13.1%, p<0.001). Repellent users were more likely to complain about the smell of the lotion (repellent users 49.5%, placebo users 44.1%, p=0.003) however this did not affect compliance (smell given as reason for non-compliance in repellent users 12.3%, placebo users 12.1%, p=0.600).

8.6 Meta-analysis of randomised controlled trials of insect repellent against malaria

A meta-analysis was carried out to assess the impact of topical insect repellents on malaria. A literature search identified four trials including the Lao PDR study that met the inclusion criteria. The average protection from malaria through the use of topical insect repellents was 33% for *P. falciparum* infection (rate ratio: 0.67, 95% CI: 0.42-1.09, p=0.11) and 35% for *P. vivax* infections (rate ratio: 0.65, 95% CI 0.18-2.34, p=0.51). However neither of these figures reached statistical significance. The variation between trials was very high, which could be explained by different background malaria rates, different compliance levels or variation between intervention formulations. There was also suggestion of publication bias that

excluded trials showing low effect from repellent. Overall the results show that topical insect repellent is not a suitable wide-scale intervention against malaria.

In comparison with other repellent trials, the results from the Lao PDR showed generally lower protection from repellent use. However the effect was within the scope of other trial results and only one of the trials showed a significant decrease in *P. falciparum* infections.

8.7 Conclusions and future work

The randomised controlled trial of 15% DEET against malaria in southern Lao PDR found no evidence of an effect on malaria. When put in the context of the results of other repellent trials, the effect is low but not significantly different from other outcomes. Overall the combined effect of repellent use from four randomised controlled trials was a 33% reduction in *P. falciparum* and a 35% reduction in *P. vivax*. These figures are much lower than the 50% effect that the trial in the Lao PDR hoped to detect. However, there have only been four fully reported trials and any attempt to compare the results is confounded by varying levels of malaria endemicity, user compliance and co-interventions.

Compliance with daily or nightly repellent use is very difficult to assess as direct observation is only practicable in a small number of participants and most of the trials used a combination of self-reported data confirmed by a small number of direct observations. There did seem to be a relationship between higher compliance and greater intervention effect. This relationship has also been suggested by a mathematical model built to explore a repellent's potential to reduce malaria. The model found that a drop in compliance from 98% to 80% would result in an even larger reduction in effect from 89% to 48% [137]. Coverage in a control programme is likely to be lower than within the highly controlled conditions of a clinical trial, meaning repellents used as a wide-scale intervention would have very little impact. In the Lao PDR compliance with use every evening was less than 60% although over 90% of participants claimed to like using the repellent and adverse reactions were rare. The most common reason for not using the lotion

was forgetfulness rather than not liking the lotion. This suggests that topical repellents are limited as an intervention tool as they require too great a behaviour change in users. Repellents applied to clothing could be a suitable alternative to protect against outdoor biting, but at present there is little data on their effectiveness and none on their efficacy to reduce disease transmission. Traditional clothing in the Lao PDR tends to leave the lower legs and feet, favourite feeding locations for mosquitoes, uncovered. Trials are required to determine if repellent clothing can protect the whole person against biting rather than just the covered skin. In addition while it has been shown that mosquitoes can be diverted from repellent users to non-users over a distance of 1m, it is not known whether mosquitoes can be diverted to another part of the body. Mosquitoes tend to prefer biting around the feet and ankles, so it would be useful to know if this can be changed by the use of repellents on the ankles.

Although *An. minimus* and *An. maculatus* were captured in entomological collections, most biting pressure came from vectors of Japanese Encephalitis (JE) rather than malaria vectors. Very little is known about JE in the Lao PDR as until recently there has been no national diagnostic programme. However the arbovirus is confirmed in neighbouring China, Thailand and Vietnam, where vaccine programmes appear to have helped reduce cases and may be on the increase in Cambodia and Myanmar, where like the Lao PDR there are no vaccine programmes or diagnostic centres [154]. This trial aimed to assess the impact of repellents on malaria, but repellents also have the potential to prevent other vector-borne diseases. As arboviruses like JE and dengue can be epidemic problems, repellents could be targeted to vulnerable populations at high-risk times. This avoids possible health risks from long-term use and might result in higher compliance as users would have to modify their behaviour for a much shorter time.

Repellents have low lethal effects in the field as insects are repelled before they come into contact with the chemical. Therefore there is much lower probability of the emergence of resistance. However if the mode of action of a repellent overlaps with an insecticide used in

the area, then repellent resistance could be driven into the population. The modes of action of repellents are not completely established, and like insecticides may vary with the chemical family. However with strongly anthropophilic vectors there may be a fitness cost associated with widespread repellent use, which could lead to the emergence of resistance. Research in this area would be required if repellents were adopted into control programmes.

The entomology studies for this trial, found that most biting in a village setting in the study area actually comes from JE vectors. Given the high ownership of pigs, amplifying intermediate hosts for the virus, and the lack of any national vaccination programme in the Lao PDR, there a real risk that JE is well established in the study area. These results argue strongly for monitoring of this potentially fatal disease in both the human population and the livestock reservoir.

There remains a real urgency to find novel control tools to address the malaria problem in the GMS in order to control the spread of artemisinin-resistance. Although LLINs work well in this region, outdoor biting means they are unlikely to be sufficient as a sole intervention method. Signs of changes in mosquito behaviour in Africa also suggest protection against outdoor biting might be needed in other areas too. Repellents can protect against outdoor biting, but further trials of topical repellent aimed at developing a wide-scale malaria control tool are not recommended based on these results. What is needed now are trials of alternative modes of repellents delivery such as impregnated clothing as well as trials that can measure the impact of repellents on other vector-borne diseases.

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Appendix A: Ethics Approvals

Ethical approval from the London School of Hygiene & Tropical Medicine

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

ETHICS COMMITTEE

APPROVAL FORM
Application number: **5471**

Name of Principal Investigator	**Nigel Hill**
Department	**Infectious and Tropical Diseases**
Head of Department	**Professor Simon Croft**

Title: **Evaluation of insect repellent to control vector-borne disease in SE Asia**

This application is approved by the Committee.

Chair of the Ethics Committee ..... T W Meade .....

Date ..... 25 March 2009 .....

Approval is dependent on local ethical approval having been received.

Any subsequent changes to the application must be submitted to the Committee via an E2 amendment form.

Approval for changes to study protocol from the London School of Hygiene & Tropical Medicine

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

ETHICS COMMITTEE

APPROVAL FORM
Application number: **A158 5471**

Name of Principal Investigator	**Professor Steve Lindsay**
Department	**Public Health and Policy**
Head of Department	**Professor Anne Mills**

Title: **Evaluation of insect repellent to control vector-borne disease in S.E. Asia**

Amendments to this application have been approved by the Ethics Committee.

Chair of the Committee..... T W Meade .....

Date .....21 May 2010.....

Approval is dependent on local ethical approval having been received.

Any subsequent changes to the application must be re-submitted to the Committee.

Ethical Clearance from Lao Ministry of Health

Lao People's Democratic Republic
Peace independence Democracy Unity Prosperity

Ministry of Health
National Ethics Committee
For Health Research (NECHR)

No. 228 /NECHR

Ethical Clearance

Project Title: Clinical Evaluation of Insect Repellent to Prevent Vector-Borne Disease Among Rural Populations on the Laos-Cambodia Border

Objectives:

- To evaluate the efficacy of DEET insect repellent to reduce malaria incidence amongst rural agricultural workers in southern Lao PDR
- To establish the effect of repellent use on the other vector borne diseases of dengue, Japanese Encephalitis (JE) and typhus

Ethical Considerations:

According to the Declaration of Helsinki, a recognized NECHR approves the protocol of this study before it is initiated. NECHR is a focal point for approval all health research to human subject activities including ethical clearance. The investigator is committed in compliance with local requirements, to inform the NECHR of any emergent problems, serious adverse reactions, or protocol amendments. Every attempt should be made to ensure confidentiality for the respondents. The data should be kept in a secure place at Population Services International (PSI) with only the researcher and supervisors are able to access the data. Participation in the researcher should be on the voluntary basis and consent should be obtained through the verbal and written consent of the respondent, final report should be submitted to NECHR and secretary committee after its completion.

Statement for Ethical Clearance:

NECHR confirms that the proposed project "**Clinical Evaluation of Insect Repellent to Prevent Vector-Borne Disease Among Rural Populations on the Laos-Cambodia Border**" has been approved. We believe that this project will contribute to a great importance of health promotion, disease prevention, health Policy, and health service in the future through the research activities.

Vientiane Capital, 16 / 06 / 2009
President, National Ethics Committee
for Health Research

Prof. Dr. Sithat INSISIENGMAY

Ethical approval from Population Services International (host organisation in Lao PDR)

Institutional Review Board (IRB)
Authorization Agreement

Name of Institution Providing IRB Review (Institution A):
London School of Hygiene & Tropical Medicine

IRB Registration: *00003028* **Federalwide Assurance (FWA)**: *00002708*

Name of Institution Relying on the Designated IRB (Institution B):
Population Services International

FWA: *00009154*

The Officials signing below agree that ***Population Services International*** may rely on the designated IRB for review and continuing oversight of its human subjects research described below: (*check one*)

(**X**) This agreement is limited to the following specific protocol(s):

Name of Research Project: *Clinical evaluation of insect repellent to prevent vector-borne disease among rural populations on the Laos-Cambodia border*

Name of Principal Investigator: *Dr Nigel Hill, London School of Hygiene and Tropical Medicine*

Sponsor or Funding Agency: *PSI Innovations Fund Grant*

The review performed by the designated IRB will meet the human subject protection requirements of Institution B's OHRP-approved FWA. The IRB at Institution A will follow written procedures for reporting its findings and actions to appropriate officials at Institution B. Relevant minutes of IRB meetings will be made available to Institution B upon request. Institution B remains responsible for ensuring compliance with the IRB's determinations and with the Terms of its OHRP-approved FWA. This document must be kept on file by both parties and provided to OHRP upon request.

Signature of Signatory Official (Institution A): T. W. Meade

Date: 14 May 2009

Print Full Name: T.W. MEADE

Institutional Title: Chair, Research Ethics Committee London School of Hygiene & Tropical Medicine

NOTE: The IRB of Institution A must be designated on the OHRP-approved FWA for Institution B.

Signature of Signatory Official (Institution B): Steven Chapman 04/08/09

Date:

Print Full Name: *Steven Chapman*

Institutional Title: *Chief Technical Officer*

Appendix B: Consent forms, information sheets, survey forms and other materials.

Consent form for participation in trial (in English and Lao)

Consent Form

Clinical evaluation of mosquito repellent

Study Co-ordinator **Vanessa Chen-Hussey**
c/o PSI Laos, House No. 268/18,
Saphanthong Tai Village, Sisattanak District,
Vientiane, Lao PDR
Mobile: 0204292769

Study Principle Investigator **Steve Lindsay**
LSHTM, Keppel Street, London, WC1E 7HT,
UK
Tel: +44 (0)207 927 2674
Fax: +44 (0)207 927 2675

I have attended the village meeting where details of the study were presented and have read the information sheet concerning this study and I understand what will be required of me and what will happen to me if I take part in it.

My questions concerning this study have been answered by the local health worker.

I understand that at any time I may withdraw from this study without giving a reason and without affecting my normal care and management

I agree to provide blood spots at the start and end of the study that can be stored for up to 12 months for diagnostic use directly associated with this study.

I agree to take part in this study

Signed ..... Date .....

Witnessed ..... Date .....

ใบสัมยา

ว่าด้วยการตกลงเข้าร่วมในการวิไจทดลองยาหากันยุง

ผู้ประสานงานการค้นคว้า (ฝ่ายลาว) แวนเนดสา เจ็น ฮาดส์เส็น
Vanessa Chen-Hussey
c/o PSI Laos, House No. 268/18, Saphanthong Tai Village, Sisattanak District, Vientiane, Lao PDR
Mobile: 0204292769

ผู้อำนวยการ และผู้ภวดสอบ การค้นคว้า (ฝ่ายอังกิด) นิแกว ฮิว
Nigel Hill
LSHTM, Keppel Street, London, WC1E 7HT, UK
โท: +44 207 927 2646
แฟกส์: +44 207 8739

ข้าพะเจ้าได้เข้าร่วมกองประชุมชั้นบ้าน
เซิ่งเพิ่นได้ให้ข้อมูนก่วกับการนำใช้ยาหากันยุง ใน โลยะทิดลองย่างละอยด
และข้าพะเจ้ายังได้รับรู้ข้อมูนเพิ่มเติมจากการอ่านใบข้อมูน ก่วก
กับการวิไจในถั้งนี้ว่าข้าพะเจ้าต้องประกอบส่วนแมวใด และจะมีหัยๆเกิดขึ้นแด่ ถ้า ข้าพะเจ้าได้เข้าร่วมในการวิไจในถั้งนี้.
บอกจากนี้ พะนักงานสาหาละบะสุกห้องถิ่นได้ตอบบันหาทุกข้อที่ข้าพะเจ้าสิงใสก่วก กับการวิไจยากันยุงในโลยะทิดลองในถั้งนี้.
ข้าพะเจ้ายังเข้าใจว่า ข้าพะเจ้าสามาดถอนตัวได้จากการวิไจทุกเวลาโดยบ่จำเป็นมีเหดผินหัยๆ และบ่มีผินสะห้อนหัยๆต่อข้าพะเจ้าเอง และ การนจัดตั้ง.
ข้าพะเจ้ายินดี และมีความเต็มใจที่จะมอบเลือดให้แก่การวิไจในถั้งนี้ ทั้งโลยะเล็มตน และโลยะสุดห้ายของการวิไจ เซิ่งยืดเลือดถั่งก่าจะสามาดเก็บไว้ได้เถิง 12 เดือน เพื่อไปนำใช้และภวดสอบโดยกิงก่วกับการวิไจในถั้งนี้.

ข้าพะเจ้ายินดีเข้าร่วมในการวิไจทิดลองการใช้ยากันยุงในถั้งนี้

ลายเส็นของผู้เข้าร่วม ..... วันที่ .....

ลายเส็นของพะยาน ..... วันที่ .....

Information sheet for participation in trial (in Lao only, English translation provided here)

Information for Volunteers

Clinical evaluation of mosquito repellent

Introduction
Mosquitoes can carry many different diseases including malaria & Dengue in many parts of Asia. People working outdoors, particularly in the evening, are at particular risk of mosquito bites. Mosquito repellents are used in many parts of the world to reduce mosquito bites. We need to find out if people using mosquito repellents can be protected from getting diseases carried by mosquitoes.

Why are we doing this study?
We know that mosquito repellents can reduce mosquito bites. However, there is very little evidence that using repellents can prevent diseases carried by mosquitoes. We think that using repellents may help reduce diseases like malaria but we need to be sure before we can use them as part of a malaria control method. This study will provide information to help us decide if repellents can be effective. The study has been approved by the ethics committee in Laos.

What is the study?
To see if repellents are effective we need to compare people using repellents with those who are not to see if fewer people get diseases like malaria. The best way to do this is to get a large number of people in different villages to take part in our study. We will then give half of these people a repellent, and the other half will get a similar liquid but that does not repel mosquitoes. We will then record how many people get diseases like malaria over the next 9 months. At the end of the study we will see if fewer people using repellent got malaria or if there was no difference.

What will happen if I take part in the study?
Everyone in the village will be given information about the study at a village meeting. Those people who would like to take part in the study will be given this information sheet and can discuss it with the local health staff who will answer any questions you have. We will also ask you some questions to be sure you are able to take part. Everyone who takes part in the study can drop out at any time without giving a reason. At the start of the study the local health worker will take a small amount of blood from the tip of your finger. This blood spot will be used in a test to see if you have malaria. Another blood spot will be taken on paper which we will dry and store to test for other mosquito carried diseases at the end of the study. You will need to give one blood drop in the same way every month for 9 more months to see if you have malaria each month during the study. If the test ever finds you have malaria we will be sure that you get the best treatment as soon as possible. Everyone taking part in the study will be given a new mosquito bed net to protect them against mosquitoes while they are in bed. We want you to remember to sleep under your net every night, but if you forget you can let us know when we visit you each month. As well as the bed net, everyone

will be given 3 bottles of liquid to rub onto their bare legs & arms every evening when the sun goes down. We will show you how to put on this liquid and there will be enough in the 3 bottles to use it every night for 1 month. Each month when we return to take the blood spot we will also give you 3 more bottles of liquid for the next month. We would like you to remember to use the liquid every evening, but if you forget you can tell us when we visit. Half of the people taking part in the study will have liquid that has a mosquito repellent, and the other half will have a liquid with no mosquito repellent. You will not know which one you have and the health worker coming each month will not know which one you have. At the end of the study we will take a final blood spot from your finger to test for malaria and one to store on paper to look for other mosquito carried diseases. We will not bring any more repellent but you will be able to keep all the bed nets we gave to you and your family as these will still be effective for several more years. Remember, if you no longer want to continue in the study you can drop out at any time without giving a reason. At the end of the study we will compare the results of the blood tests to see if there is a difference between those using the repellent of those who did not. After the tests are complete we will store no samples and we will only keep records with numbers, not your names.

Will it be painful and is it safe?
When the health worker takes the blood spot from your finger it will sting for a little while. Only one or 2 small spots of blood are taken and this will be very quick. We will give you a small tissue to keep the finger clean. This way of taking blood drops to test for malaria is exactly the same as the one used in your village by the health workers and in your local clinic a hospital, it is very easy, very fast and very safe.

The bed nets we will give you are the same as those used in Laos and many other parts of the world where there are mosquitoes carrying malaria. They are safe and will help prevent you get bitten at night in bed.

The liquid mosquito repellent we will give you is a very common product sold in most Countries in the world including America and Europe. It is used by many people around the world and sold in most Countries. It has passed safety testing in all Countries and is already sold in Laos. A very few people may be allergic to the lotion, and we will ask you if you have any problems. If you use it and you think it is making you unwell, or if you get red skin or itching you should stop using it and tell the health worker or study manager (contact details below). It is very rare for people to have problems using mosquito repellents, but you must always follow the instructions on the bottle or given to you by the health worker at the start of the study.

How to contact us

Your local health worker is ..... – Contact ;
The study manager is Vanessa Chen Hussey – Mobile: 020 4292769

ຂໍ້ມູນກ່ຽວກັບການວິໄຈທົດລອງການໃຊ້ຢາຫາກັນຍຸງ
ສຳລັບອາສາສະມັກ

ຄຳນຳ

ຍຸງສາມາດນຳພາເອົາເຊື້ອພະຍາດມາສູ່ຄົນເຮົາໄດ້ຫຼາຍຊະນິດ ເຊັ່ນ ມາລາເລຍ ແລະພະຍາດໄຂ້ເຊື່ອງຈະພົບເຫັນຢູ່ໃນຫຼາຍໆປະເທດທົ່ວໂລກໃນທະວີບອາຊີ. ສ່ວນຫຼາຍແມ່ນເກີດກັບກຸ່ມຄົນທີ່ມີອາຊີບເຮັດວຽກຕອນກາງຄືນ ແລະເຮັດວຽກຢູ່ນອກອາຄານ ຫຼືກາງເດີ່ນ, ດັ່ງນັ້ນ ແມ່ນມີຄວາມສ່ຽງຕໍ່ການຖືກຍຸງກັດ. ສະນັ້ນຢາຫາກັນຍຸງແມ່ນໄດ້ຖືກນຳໃຊ້ຢ່າງກວ້ວງຫຼາຍເພື່ອຫຼຸດຜ່ອນບໍ່ໃຫ້ຍຸງກັດ. ດັ່ງນັ້ນ ພວກເຮົາຢາກວິໄຈວ່າ ການນຳໃຊ້ຢາຫາດັ່ງກ່າວ ແມ່ນສາມາດປົກປ້ອງຄົນ ທີ່ໄດ້ນຳ ໃຊ້ຢາຕິວນີ້ຈາກພະຍາດທີ່ເກີດຈາກຍຸງນຳມາໄດ້ ຫຼືບໍ່.

ເປັນຫຍັງພວກເຮົາຈຶ່ງຢາກເຮັດການວິໄຈດັ່ງກ່າວນີ້

ພວກເຮົາຮູ້ແລ້ວວ່າ ຢາກັນຍຸງນີ້ ແມ່ນສາມາດຫຼຸດຜ່ອນການກັດຂອງຍຸງໄດ້ ແຕ່ວ່າ ພວກເຮົາບໍ່ມີຫຼັກຖານໜຸ່ງໜ່ວ່າ ການນຳໃຊ້ຢາຫາກັນຍຸງນີ້ສາມາດປົກປ້ອງຜູ້ນຳໃຊ້ຈາກ ເຊື້ອພະຍາດທີ່ເກີດມານຳ ຍຸງໄດ້ ຫຼືບໍ່ ດັ່ງນັ້ນ ພວກເຮົາຄິດວ່າການນຳໃຊ້ຢາກັນຍຸງອາດຈະສາມາດ ຫຼຸດຜ່ອນພະຍາດທີ່ມານຳ ຍຸງໄດ້ ເຊັ່ນ ມາເລເລຍ ແຕ່ພວກເຮົາຕ້ອງການຄວາມແນ່ໃຈວ່າຢາດັ່ງ ກ່າວນີ້ ແມ່ນນຳໃຊ້ໄດ້ຜົນຈິງ ກ່ອນທີ່ເຮົາຈະນຳໃຊ້ມັນເຂົ້າໃນການຄວບຄຸມເຊື້ອໄຂ້ມາລາເລຍ. ເຊິ່ງຜົນການວິໄຈດັ່ງກ່າວຈະ ຊ່ວຍໃຫ້ພວກເຮົາຮູ້ວ່າ ຢາກັນຍຸງນີ້ ແມ່ນໃຊ້ໄດ້ຜົນຈິງ. ການວິໄຈນີ້ ແມ່ນໄດ້ຮັບອະນຸຍາດຈາກ ຄະນະກຳມະການທາງດ້ານຈັນຍາບັນຂອງລາວ.

ການວິໄຈດັ່ງກ່າວນີ້ແມ່ນເປັນແນວໃດ

ເພື່ອຢາກຮູ້ວ່າ ການໃຊ້ຢາກັນຍຸງນີ້ແມ່ນໄດ້ຜົນ ຫຼືບໍ່ໄດ້ຜົນນັ້ນ ພວກເຮົາຈະທຳການປຽບທຽບ ລະຫວ່າງ ກຸ່ມຄົນທີ່ໄດ້ໃຊ້ຢາຫານີ້ ແລະກຸ່ມຄົນທີ່ບໍ່ໄດ້ໃຊ້ຢາດັ່ງກ່າວນີ້ເພື່ອ ສັງເກດວ່າຈະມີຈຳ ນວນຄົນ ທີ່ຕິດເຊື້ອພະຍາດຈາກຍຸງ ເຊັ່ນມາລາເລຍ ຫຼຸດລົງ ຫຼືບໍ່. ພວກເຮົາຈະໄດ້ທຳການຄັດເລືອກຫຼາກ ຫຼາຍ ໝູ່ບ້ານທີ່ມີປະຊາກອນຈຳນວນຫຼາຍຢູ່ໃສ ເພື່ອປະກອບສ່ວນເຂົ້າໃນການວິໄຈໃນຄັ້ງນີ້. ຈາກນັ້ນ ພວກເຮົາກໍ່ຈະແຈກຢາຍຢາກັນຍຸງໃຫ້ແກ່ ກຸ່ມ ທີ 1ໄດ້ທົດລອງໃຊ້ ແລະແຈກຢາຍຢາຫາທີ່ຄ້າຍ ຄືກັນ ແຕ່ບໍ່ສາມາດກັນຍຸງໄດ້. ຫຼັງຈາກນັ້ນ 9 ເດືອນ ພວກເຮົາຈະນັບຫຶກວ່າມີຈຳ ນວນຈັກຄົນທີ່ຕິດ ເຊື້ອພະຍາດຈາກຍຸງ ເຊັ່ນມາເລເລຍ ແລະພວກເຮົາຈຶ່ງຈະຮູ້ວ່າໃນການວິໄຈຄັ້ງນີ້ຈະມີຈຳນວນຄົນ ທີ່ມີເຊື້ອມາເລເລຍຫຼຸດລົງໂດຍການ ໃຊ້ຢາກັນຍຸງ ຫຼືບໍ່.

ຈະມີຫຍັງເກີດຂຶ້ນກັບຂ້ອຍແດ່ ຖ້າຂ້ອຍໄດ້ເຂົ້າຮ່ວມໃນການວິໄຈໃນຄັ້ງນີ້

ທຸກໆຄົນໃນໝູ່ບ້ານຈະໄດ້ຖືກແຈ້ງ ກ່ຽວກັບການວິໄຈໃນຄັ້ງນີ້ ໂດຍຜ່ານກອງປະຊຸມຂັ້ນບ້ານ. ສ່ວນບຸກຄົນໃດທີ່ສົນໃຈຢາກເຂົ້າຮ່ວມໃນການວິໄຈກໍ່ຈະໄດ້ຮັບໃບຂໍ້ມູນສະບັບນີ້ ແລະທ່ານສາ ມາດໄຂ່ລົມກັບພະນັກງານສາທາລະນະສຸກທ້ອງຖິ່ນໄດ້ ເຊິ່ງເພິ່ນຈະເປັນຜູ້ຕອບຄຳຖາມທຸກຂໍ້ທີ່ ທ່ານສົງໃສກ່ຽວກັບການວິໄຈໃນຄັ້ງນີ້. ນອກຈາກນີ້ ພວກເຮົາຍັງມີຄຳຖາມສຳລັບທ່ານທີ່ສົນໃຈ

ເຂົ້າຮ່ວມໃນ ການວິໄຈຫົດລອງຢາຫາກັນຍຸງນີ້ ເພື່ອເບິ່ງວ່າທ່ານມີເງື່ອນໄຂພຽງພໍທີ່ຈະປະກອບ ສ່ວນເຂົ້າໃນການວິໄຈໃນຄັ້ງນີ້. ທ່ານໃດທີ່ໄດ້ເຂົ້າຮ່ວມໃນການວິໄຈໃນຄັ້ງນີ້ ທ່ານສາມາດຖອນ ໂຕໄດ້ຕະຫຼອດເວລາ ໂດຍບໍ່ຈຳເປັນຕ້ອງໃຫ້ເຫດຜົນໃດໆທັ້ງສິ້ນ.

ໃນເບື້ອງຕົ້ນຂອງການວິໄຈ ພະນັກງານສາທາລະນະສຸກຫ້ອງຖິ່ນກໍ່ຈະໄດ້ທຳການເຈາະເລືອດ ຈາກນິ້ວຂອງທ່ານ 2 ຫາ3 ຢົດ. ຢົດທຳອິດ ແມ່ນເພື່ອກວດເບິ່ງວ່າທ່ານມີເຊື້ອພະຍາດໄຂ້ມາເລ ເລຍຫຼືບໍ່ ສ່ວນຢົດທີ2 ພວກເຮົາຈະເກັບຮັກສາໄວ້ໃນໄລຍະສຸດທ້າຍຂອງການວິໄຈ ໂດຍການ ຢອດໃສ່ເຈ້ຍ ແລະເຮັດໃຫ້ແຫ້ງ ເພື່ອ ກວດເບິ່ງວ່າທ່ານມີເຊື້ອພະຍາດອື່ນອີກບໍ່ທີ່ມານຳຍຸງ,

ທ່ານຍັງຕ້ອງໄດ້ຖືກເຈາະເລືອດທຸກໆເດືອນ ເປັນເວລາ 9 ເດືອນ ເພື່ອກວດເບິ່ງວ່າທ່ານມີເຊື້ອໄຂ້ ມາເລເລຍຫຼືບໍ່ຕະຫຼອດໄລຍະການວິໄຈ. ພວກເຮົາຮັບປະກັນວ່າ ທ່ານຈະໄດ້ຖືກປິ່ນປົວຢ່າງຖ່ວງທີ ຖ້າທ່ານມີເຊື້ອໄຂ້ດັ່ງກ່າວ.

ສຳລັບທ່ານທີ່ໄດ້ເຂົ້າຮ່ວມໃນການວິໄຈໃນຄັ້ງນີ້ແລ້ວ ທ່ານຈະໄດ້ຮັບມຸ້ງຟີຣອັນໜຶ່ງເພື່ອປ້ອງກັນ ຈາກການຍຸງກັດໃນເວລາທີ່ທ່ານນອນ ແລະ ພວກເຮົາຂໍແນະນຳໃຫ້ທ່ານນອນຢູ່ໃນມຸ້ງທຸກໆຄືນ ແຕ່ຫາກວ່າທ່ານ ລືມການມຸ້ງໃນເວລານອນ ທ່ານກໍ່ສາມາດບອກພວກເຮົາໄດ້ໃນເວລາ ທີ່ພວກ ເຮົາລົງໄປຢ້ຽມຢາມທ່ານໃນທຸກໆເດືອນ. ນອກຈາກທ່ານຈະໄດ້ມຸ້ງແລ້ວ ທ່ານຍັງຈະໄດ້ຮັບຢາ ຫາກັນຍຸງ 3 ຂວດ ເພື່ອ ໃຫ້ທ່ານເອົາໄປຫາແຂວ ແລະຂາທຸກໆຄືນ ຫຼັງຈາກຕາເວັນຕົກດິນແລ້ວ. ພ້ອມກັນນັ້ນ ພວກເຮົາກໍ່ ຈະແນະນຳວິທີການໃຊ້ຢາຫາກັນຍຸງໃຫ້ທ່ານຮູ້. ຢາຫາກັນມີທັງໝົດ 3 ຂວດ ຊຶ່ງກຸ້ມໄດ້ປະມານ 1 ເດືອນໂດຍການຫາຢາດັ່ງກ່າວນີ້ທຸກໆຄືນ ແລະ ໃນທຸກໆເດືອນ ຕະຫຼອດໄລຍະການວິໄຈທ່ານຈະໄດ້ຮັບຢາຫາກັນຍຸງ 3 ຂວດ ໃຫມ່ ໂດຍທີ່ພວກເຮົາຈະເອົາໃຫ້ ທ່ານໃນເວລາທີ່ພວກເຮົາລົງໄປຢ້ຽມຢາມ ແລະເກັບກຳເລືອດຂອງ ພວກທ່ານໃນແຕ່ລະເດືອນ ແລະ ພວກເຮົາຂໍແນະນຳໃຫ້ທ່ານຫາຢາດັ່ງກ່າວນີ້ທຸກໆຄືນ ແຕ່ຫາກວ່າທ່ານ ລືມຫາທ່ານກໍ່ສາມາດ ບອກ ພວກເຮົາໄດ້ໃນເວລາ ທີ່ພວກເຮົາລົງໄປຢ້ຽມຢາມທ່ານໃນທຸກໆເດືອນ.

ທ່ານທີ່ໄດ້ເຂົ້າຮ່ວມໃນການວິໄຈຄັ້ງນີ້ ຈະຖືກແບ່ງອອກເປັນ 2 ສ່ວນ: ສ່ວນທີ1 ພວກເຮົາຈະແຈກ ຢາຍຢາຫາກັນຍຸງໃຫ້ ແລະສ່ວນທີ2 ຈະຖືກແຈກຢາຍຢາທີ່ຄ້າຍຄືກັນ ແຕ່ບໍ່ແມ່ນຢາຫາກັນຍຸງ.

ທ່ານຈະບໍ່ຮູ້ວ່າທ່ານຈະໄດ້ຢາຊະນິດໃດ ແລະການລົງມາຢ້ຽມຢາມຂອງພະນັກງານ ສາທາລະນະ ສຸກຫ້ອງຖິ່ນໃນທຸກເດືອນນັ້ນ ເພື່ນກໍ່ບໍ່ຮູ້ເຊັ່ນກັນວ່າ ທ່ານກຳລັງໃຊ້ຢາຊະນິດໃດຢູ່.

ໃນໄລຍະສຸດທ້າຍຂອງການວິໄຈ ພວກເຮົາຈະທຳການເຈາະເລືອດຂອງທ່ານເປັນຄັ້ງສຸດທ້າຍ ເພື່ອກວດເບິ່ງວ່າທ່ານມີເຊື້ອພະຍາດໄຂ້ມາເລເລຍ ຫຼື ບໍ່ ແລະອີກເມັດໜຶ່ງ ແມ່ນ ພວກເຮົາຈະ ເກັບຮັກ ສາໄວ້ໂດຍການຢອດໃສ່ເຈ້ຍ ແລະເຮັດໃຫ້ແຫ້ງ ເພື່ອກວດເບິ່ງວ່າທ່ານ ມີເຊື້ອພະຍາດອື່ນ ອີກບໍ່ທີ່ມານຳຍຸງ. ຫຼັງຈາກນັ້ນ ພວກເຮົາຈະຍຸດຕິການແຈກຢາຍຢາຫາກັນຍຸງ ແຕ່ມຸ້ງທີ່ພວກເຮົາ ໄດ້ທຳການແຈກຢາຍໄປແລ້ວນັ້ນ ແມ່ນທ່ານສາມາດເກັບໄວ້ໄດ້ ເພາະວ່າ ພວກເຮົາເຊື່ອວ່າມັນສາ ມາດນຳໃຊ້ໄດ້ອີກຫຼາຍປີ ແລະ ມັນຈະເປັນປະໂຫຍດຕໍ່ທ່ານ ແລະຄອບຄົວຂອງທ່ານ. ແຕ່ທ່ານ ຢ່າລືມວ່າ ທ່ານສາມາດຖອນໂຕໄດ້ຕະຫຼອດເວລາ ໂດຍບໍ່ຈຳ ເປັນຕ້ອງບອກເຫດຜົນໃດໆທັ້ງສິ້ນ.

ໃນໄລຍະສຸດທ້າຍຂອງການວິໄຈ ພວກເຮົາຈະທຳການປຽບທຽບຜົນການກວດເລືອດຂອງກຸ່ມຄົນທີ່ ໄດ້ນຳໃຊ້ຢາຫາກັນຍຸງ ແລະກຸ່ມຄົນທີ່ບໍ່ໄດ້ໃຊ້ຢາດັ່ງກ່າວ ເພື່ອເບິ່ງວ່າມັນມີຄວາມແຕກຕ່າງກັນບໍ່ ລະຫວ່າງ 2 ກຸ່ມນີ້. ຫຼັງຈາກການກວດເລືອດສຳເລັດຮຽບຮ້ອຍແລ້ວ ພວກເຮົາກໍ່ຈະບໍ່ເກັບຮັກສາຕົວ

ຢ່າງເລືອດຂອງທ່ານໄວ້ ພວກເຮົາມີພຽງແຕ່ຮັກສາຍົດບັນທຶກ ແລະຕົວເລກເທົ່ານັ້ນ ນັ້ນໝາຍ ຄວາມ ວ່າ ຊື່ຂອງທ່ານຈະບໍ່ປາກົດໃນບົດລາຍງານຂອງການວິໄຈໃນຄັ້ງນີ້.

<u>ການວິໄຈໃນຄັ້ງນີ້ ແມ່ນເຈັບ ແລະປອດໄພບໍ່</u>

ເມື່ອພະນັກງານສາທາລະນະສຸກເຈາະເອົາເລືອດຈາກນິ້ວມືຂອງທ່ານແລ້ວ ທ່ານອາດຈະຮູ້ສຶກເຈັບ ໜ້ອຍໜຶ່ງ ແຕ່ມີພຽງ 1 ຫາ 2 ຢົດເລືອດເທົ່ານັ້ນ ແລະມັນເປັນຂະບວນການທີ່ໄວ ແລະພວກເຮົາຈະ ໃຫ້ສຳລີທີ່ຜ່ານການຂ້າເຊື້ອພະຍາດແລ້ວ ແກ່ທ່ານເພື່ອທຳຄວາມສະອາດນິ້ວມືຂອງທ່ານ. ວິທີການເຈາະ ເລືອດດັ່ງກ່າວນີ້ ແມ່ນໄດ້ຖືກນຳໃຊ້ຕາມສູນອານາໄມ ແລະໂຮງໝໍທົ່ວໄປ ແລະມັນເປັນວິທີທີ່ງ່າຍ ໄວ ແລະປອດໄພຫຼາຍ.

ສ່ວນມຸ້ງທີ່ພວກເຮົາຈະມອບໃຫ້ທ່ານນັ້ນ ແມ່ນປະເພດດຽວກັນກັບທີ່ຖືກນຳໃຊ້ຢ່າງແພຫຼາຍຢູ່ໃນ ລາວ ແລະຫຼາຍປະເທດທົ່ວໂລກ ຊຶ່ງເປັນເຂດທີ່ມີພະຍາດໄຂ້ມາເລເລຍລະບາດຢູ່. ມຸ້ງປະເພດນີ້ ແມ່ນປອດໄພ ແລະສາມາດປ້ອງກັນພວກທ່ານຈາກການຖືກຍຸງກັດໄດ້ໃນເວລາກາງຄືນ.

ສ່ວນຢາຫາກັນຍຸງທີ່ພວກເຮົາຈະແຈກຍາຍໃຫ້ພວກທ່ານນັ້ນ ແມ່ນສະລິດຕະພັນອັນດຽວກັນ ທີ່ຖືກ ນຳໃຊ້ ແລະຂາຍໃນຫຼາຍໆປະເທດ ເຊັ່ນອາເມລິກາ. ເນື່ອງຈາກວ່າ ຢາດັ່ງກ່າວນີ້ໄດ້ຖືກໃຊ້ຢ່າງ ແພຫຼາຍ ແລະວາງຈຳໜ່າຍໃນຫຼາຍໆປະເທດ, ມັນໄດ້ຜ່ານການກວດສອບຫາໆ ດ້ານຄວາມປອດ ໄພຈາກປະເທດເຫຼົ່ານັ້ນ ແລະຢາຫາກັນຍຸງດັ່ງກ່າວນີ້ມີວາງຂາຍແລ້ວໃນລາວ.

ອາການແພ້ຢາຫາກັນຍຸງນີ້ ແມ່ນຄົນຈຳໜ້ອຍທີ່ຈະມີອາການແພ້ຢາດັ່ງກ່າວ ແຕ່ພວກເຮົາກໍ່ຈະ ສອບຖາມພວກທ່ານກ່ຽວກັບອາການແພ້ຢາກ່ອນຈະແຈກຍາຍໃຫ້, ແຕ່ຫາກວ່າຖ້າທ່ານໃຊ້ຢາ ຫາກັນຍຸງແລ້ວ ທ່ານຮູ້ສຶກວ່າ ມັນເຮັດໃຫ້ທ່ານຮູ້ສຶກບໍ່ສະບາຍ ມີພືນຫຼືຕຸ່ມແດງເກີດຂຶ້ນ ຫຼືມີອາກັນຄັນ ທ່ານຄວນຈະຢຸດໃຊ້ຢາດັ່ງກ່າວນັ້ນທັນທີ ແລ້ວລາຍງານໃຫ້ພະນັກງານສາທາລະນະ ສຸກຮູ້ ຫຼືຕິດຕໍ່ຫາຜູ້ຈັດການການວິໄຈ(ຝ່າຍລາວ) ເຊິ່ງລາຍລະອຽດການຕິດຕໍ່ ແມ່ນຢູ່ຂ້າງລຸ່ມນີ້. ອາການແພ້ຢາຫາກັນຍຸງດັ່ງກ່າວນີ້ ແມ່ນມີໂອກາດທີ່ຈະເກີດຂຶ້ນໄດ້ໜ້ອຍ ແຕ່ພວກທ່ານ ຕ້ອງປະ ຕິບັດຕາມຄຳແນະນຳໃນການໃຊ້ຢາຫາກັນຍຸງ ດັ່ງກ່າວຢ່າງເຄັ່ງຄັດ ເພື່ອຫຼີກລ້ຽງການແພ້ຢາ ຄຳແນະນຳດັ່ງກ່າວ ຈະມີຢູ່ທາງດ້ານຫຼັງຂອງຊອງຢາ ແລະປະຕິບັດຕາມຄຳແນະນຳຈາກ ພະນັກ ງານສາທາລະນະສຸກທີ່ໄດ້ແນະນຳວິທີການໃຊ້ໃຫ້ກັບທ່ານໃນໄລຍະເລີ່ມຕົ້ນຂອງການວິໄຈ.

<u>ລາຍລະອຽດການຕິດຕໍ່</u>

ພະນັກງານສາທາລະນະສຸກທ້ອງຖິ່ນຂອງທ່ານ ແມ່ນ ທ່ານ

ຜູ້ຈັດການການວິໄຈ ແມ່ນ ທ່ານ ນາງ **ແວນເດດສຸດາ ເຈັນ ຮາດສ໌ເຊັ່ນໄທ 020 4292 769**

Consent form for participation in human landing catches (in English and Lao)

Project Informed Consent form for Human Landing Catch Collector

I,_____(name of collector) agree to participate in the following study: Survey of local species of anthropophilic mosquitoes and the effectiveness of di-ethyl toluamide (DEET) as a repellent for early evening hours. I agree that I will participate as a landing catch for collecting mosquitoes and the repellent effects of DEET.

I have been given the opportunity to ask questions concerning this study. Any such questions have been answered to my full satisfaction. Should any further questions arise concerning this study I may contact Sarah DeRaedt or Vanessa Chen-Hussey. I understand that I may revoke this consent at any time without penalty or loss of pay.

_____

Signature of participant Date

ID number _____

_____

Fieldworkers initials Date

Information sheet for participation in trial (in Lao only, English translation provided here)

Information for Volunteers

Study of Repellent Efficiency

Survey of local species of anthropophilic mosquitoes and the effectiveness of di-ethyl toluamide (DEET) as a repellent for early evening hours. Masters' project thesis. Sarah DeRaedt

Introduction

Mosquitoes can carry many different diseases including malaria and Dengue in many parts of Asia. People working outdoors, particularly in the evening, are at particular risk of mosquito bites. We need to find out if people using different concentrations of mosquito repellents will be protected from getting diseases carried by mosquitoes.

Why Are We Doing This Study?

We know that mosquito repellents can reduce mosquito bites. We think that different strengths (concentrations) of repellents will last different times and reduce biting differently. This study will tell us how long different strengths of repellents last and how much they reduce biting. This study has been approved by the Lao Ministry of Health National Ethics Committee for Health Research.

What Is The Study?

In order to test the different strengths of repellents we need to put them on people. Human landing catches are the best way to test the repellents as they are as close to real life as possible. We will give each collector a different strength of repellent or similar liquid which does not repel mosquitoes each night for 16 nights. It is important that you and we do not know which strength of repellent or non-repellent you have been given. This makes sure that the study gets rid of as much human bias as possible. The number of mosquitoes caught at the end of the study will show us the strength of each repellent.

What Will Happen If I Take Part In This Study?

Those people who want to take part in this study will be given this information sheet and can discuss it with the local health staff who will answer any questions you have. We will also ask you some questions to see if you are able to take part. Anyone who takes part in the study can stop at any time without giving a reason. At 4:30 PM each evening of the study you will be given a place to sit and a liquid to rub on your bare arms and legs. We will show you how to put on this liquid. You will not know which liquid has been given and the entomology trial supervisor giving you the liquid will not know which one you have. You will also be given a light and a tool for catching the mosquitoes called and aspirator. When you catch a mosquito you will put it into a container for us to count later. This catching will go on for 50 minutes with a ten minute break from 5 PM until 11 PM. At every ten minute break we will collect the

containers and give you new ones. At the end of the study a local health worker will take a small amount of blood from the tip of your finger. This blood will be used in a test to see if you have malaria. Remember, if you no longer want to continue in the study you can drop out at any time without giving a reason. When the study is finished we will only keep records with numbers not your names.

Will It Be Painful And Is It Safe?

There may be some pain if you get bitten by a mosquito before it is caught. When the blood from your finger gets taken it will sting for a little while. Only 1 or 2 small drops of blood are taken and this will be very quick. We will give you a small tissue to keep your finger clean. This way of taking blood drops to test for malaria is exactly the same as the one used in your village by the health workers and in your local clinic at hospital, it is very easy, very fast and very safe. The blood test will tell us if you have gotten malaria during the study, but if you feel like you have a fever, headache, or tiredness up to 15 days after the study finishes or at any point during the study, you should let us know or go to your local clinic for testing and you will be given medicine. The liquid mosquito repellent we will give you is a very common product sold in most Countries in the world including America and Europe. It is used by many people around the world and sold in most Countries. It has passed safety testing in all Countries and is already sold in Laos. A very few people may be allergic to the lotion, and we will ask you if you have any problems. If you use it and you think it is making you unwell, or if you get red skin or itching you should stop using and tell the health worker or study manager (contact details below). It is very rare for people to have problems using mosquito repellents, but you must always follow the instructions on the bottle or given to you by the health worker at the start of the study.

Will I Be Paid?

If you are able to take part in the study you will be paid 40,000 Kip and evening for 16 evenings totaling 640,000 Kip.

How to Contact Us

The study manager is Vanessa Chen Hussey – mobile: 020 4292769

English language baseline survey form (page 1/3)

Part 1: Basic Identification Information

1.	Interviewer Name	
2.	Date	
3.	Province	
4.	District	
5.	Village	
6.	Name of Head of Household	
7.	Household Number	
8.	Group (258/305)	

Part 2: Questions to Head of Household Only

9.	How many people are there in your household?		nopeople
10.	What is your main occupation?		occupation
11.	How many years of education have you had?		education
12.	What is your house made from? (Circle)	Bamboo (1) Mesh & leaves (2) Wood (3) Brick (4) Other _____	housemat
13.	What is the roof of your house made from? (Circle)	Bamboo (1) Tile (2) Thatch (3) Wood (4) Metal (5) Other _____	roofmat
14.	What type of electricity supply do you have? (Circle)	None (0) Continuous power supply (1) Evening power supply (2) Own generator (3) Other_____	electric

English language baseline survey form (page 2/3)

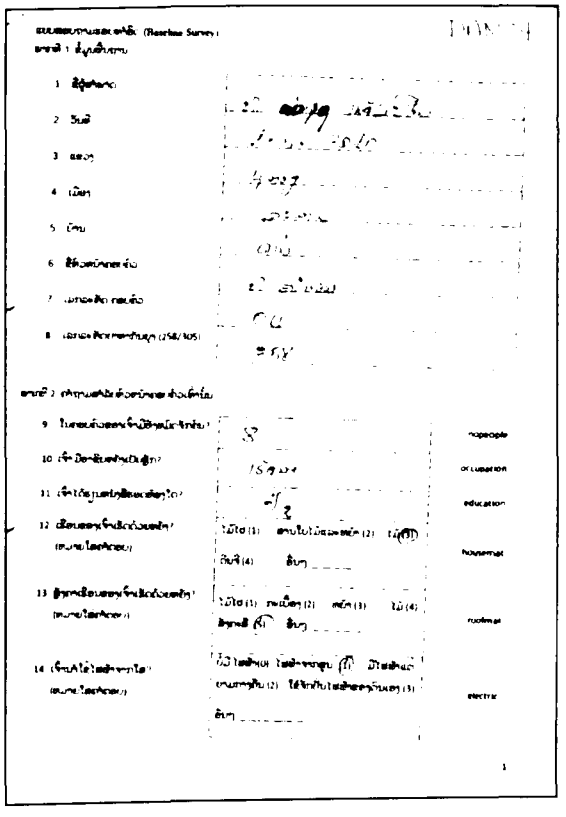
15. How is your water supplied?	Pipe (1) Well/pump (2) Spring/Stream (3) Lake/River (4)	water
16. What type of cooking fuel do you use?	Wood fire (1) Charcoal (2) Gas (3)	fuel
17. Do you own a motorcycle? Yes (1), No (0)		mbike
18. Do you own a tractor? Yes (1), No (0)		tractor
19. Do you own a radio? Yes (1), No (0)		radio
20. Do you own a television? Yes (1), No (0)		tv
21. How many insecticide treated bed nets are there in your house?		itn
22. Bed net ownership verified by interviewer Yes (1), No (0)		itnverify
23. Do you use other types of personal protection against mosquito bites? (Circle)	Untreated nets (1) Coils (2) Burning a fire (3) Burning herbs (4) Insecticide spray (5) Other_____	persprotec
24. How many animals do you own?	Buffalo	buffalo_no
	Pigs	pig_no
	Goats	goat_no
	Chickens	chick_no
	Dogs	dog_no
25. Where are they kept over night? Out of village (1), By house (2) Under house (3), Inside house (4) Free roaming (5), Other (state)	Buffalo	buffalo_locatio
	Pigs	pig_location
	Goats	goat_location
	Chickens	chick_location
	Dogs	dog_location

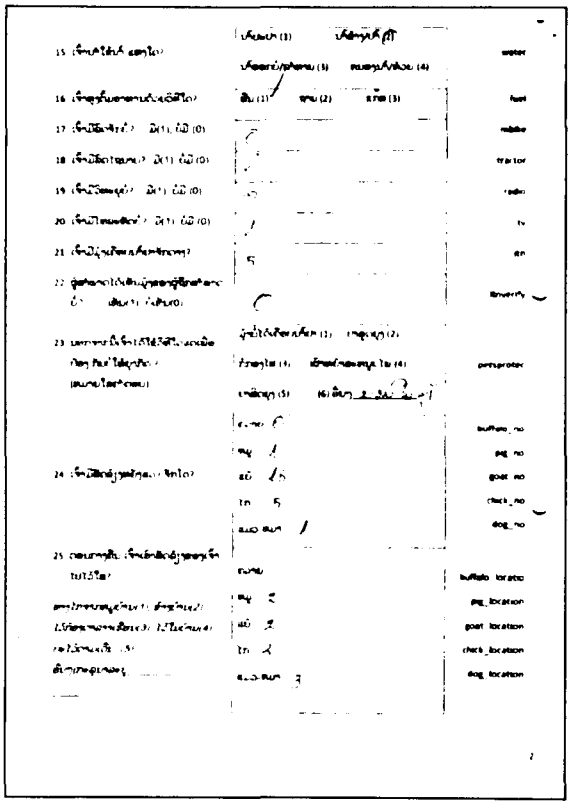
English language baseline survey form (page 3/3)

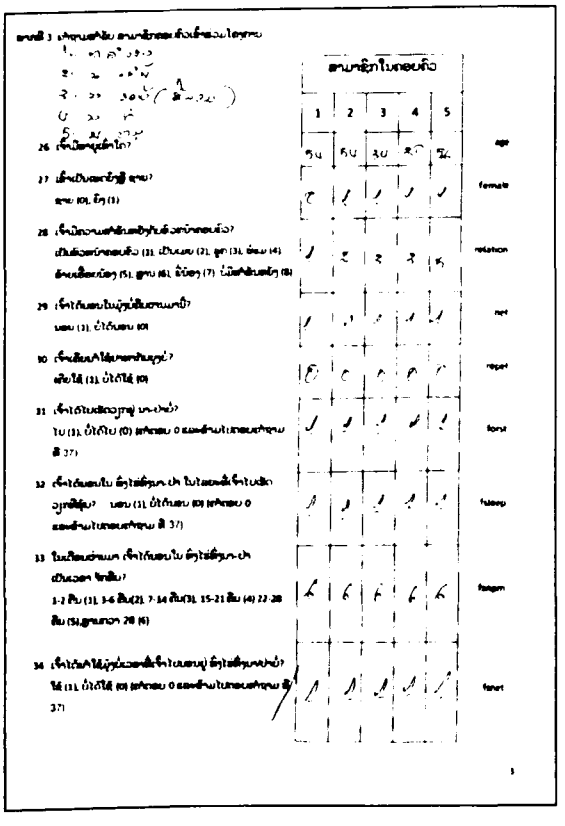
Part 3: Questions to ALL participants

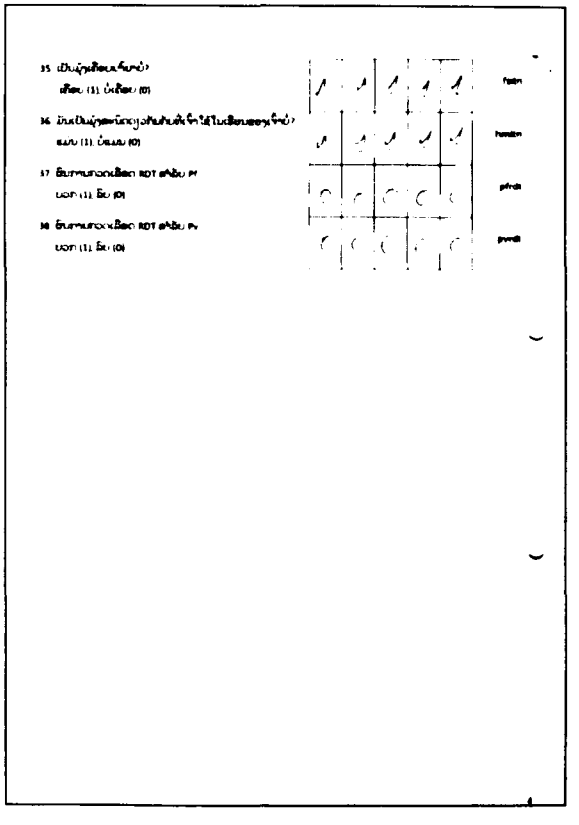
	Household Member					
	1	2	3	4	5	
26. How old are you?						age
27. Are you male or female? Male (0), Female (1)						female
28. What relation are you to the head of the household? Head (1), Spouse (2), Child (3), Parent (4), Sibling (5), Grandchildren (6), Other relative (7), Non-relative (8)						relation
29. Did you sleep under a bed net last night? Yes (1), No (0)						net
30. Do you use any kind of insect repellent? Yes (1), No (0)						repel
31. Do you work in fields or forests? Yes (1), No (0 & Go to Question 37)						forst
32. Do you sleep in the fields/forest when working there? Yes (1), No (0 & Go to Question 37)						fsleep
33. How many nights last month do you sleep there? 1-2 Nights (1), 3-6 Nights(2), 7-14 Nights (3), 15-21 Nights (4) 22-28 Nights (5), More than 28 nights (6)						fsnpm
34. Do you use a bed net when sleeping in the fields/forest? Yes (1), No (0 & Go to Question 37)						fsnet
35. Is it an insecticide treated net? Yes (1), No (0)						fsitn
36. Is it the same bed net that you use at home? Yes (1), No (0)						hmitn
37. RDT results for Pf Positive (1), Negative (0)						pfrdt
38. RDT results for Pv Positive (1), Negative (0)						pvrdt

Lao language baseline survey form (completed example)







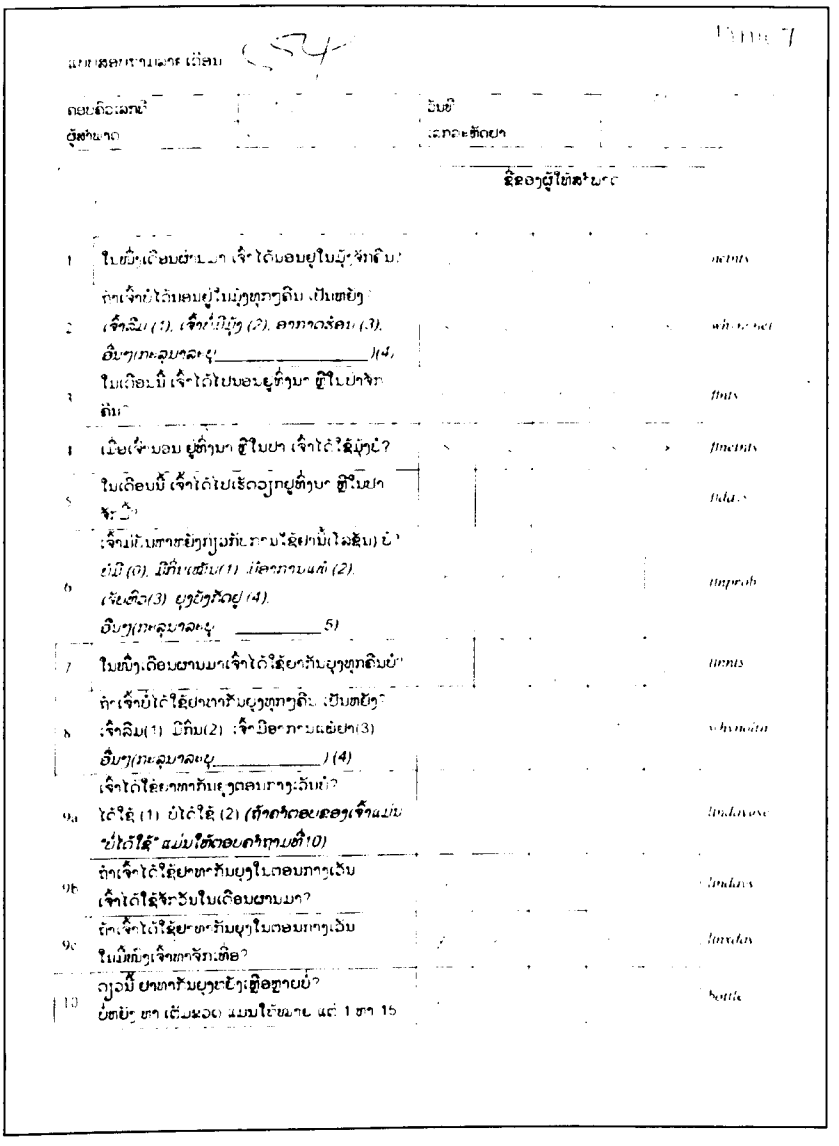


English language monthly follow-up form

Household		Date	
Interviewer		Group	

		Participant ID					
1	How many nights did you sleep under a bed net this month?						*netnts*
2	If did not sleep under bed net every night, why not? *Not applicable (99), Forgot (1), Did not have a net (2), Too hot (3), Other (state)*						*whynonet*
3	How many nights did you sleep in the fields/forest this month?						*ffnts*
4	When you slept in the fields/forest did you use a bed net? *Yes (1), No (0)*						*ffnetnts*
5	How many days did you work in the fields/forest this month?						*ffdays*
6	Did you have any problems using the lotion? *No problems (0), Bad smell (1), Allergy (2), Headache (3), Does not stop bites (4), Other (state)*						*ltnprob*
7	How many evenings did you use the lotion?						*ltnnts*
8	If NO, why did you not use the lotion? *Forgot (1), Smell (2), Allergy (3), Other (state)*						*whynoltn*
9a	Did you use the lotion during the day? *Yes (1), No (0)*						*ltndayuse*
9b	If lotion used during the day, how many days?						*ltndays*
9c	If lotion used during the day, how many times per day?						*ltnxday*
10	How much lotion is left? *None (0) to All (15)*						*bottle*
10	RDT results for *Pf* *Positive (1), Negative (0)*						*RDTPf*
11	RDT results for *Pv* *Positive (1), Negative (0)*						*RDTPv*
12	Did you have a fever this month? *None (0), One (1), More than one (2)*						*fever*
13	If had a fever, did you have an RDT? *Yes (1), No (0)*						*feverRDT*
14	What treatment did you receive? *None (0), CQ (1), ACT (2), Received treatment but no sure which (3)*						*fevertmt*

Lao language monthly follow-up form (completed example)



ແບບສອບຖາມລາຍເດືອນ

11	ຜົນການກວດໂຣກມາແລເລຍ (RDT) ສຳລັບ Pf ຜົນບວກ(1), ຜົນລົບ (0)						*RDTPf*
12	ຜົນການກວດໂຣກມາແລເລຍ (RDT) ສຳລັບ Pv ຜົນບວກ(1), ຜົນລົບ (0)						*RDTPv*
13	ເຈົ້າໄດ້ເປັນໄຂ້ບໍ່ໃນໜຶ່ງເດືອນຜ່ານມານີ້? ບໍ່ເຄີຍ (0), ເຄີຍໜຶ່ງ (1), ຫຼາຍກ່ວາໜຶ່ງເທື່ອ (2)						*fever*
14	ຖ້າເຈົ້າເປັນໄຂ້ ທານໄດ້ກວດຫາເຊື້ອໂຣກມາແລເລຍ ບໍ? ໄດ້ກວດ (1), ບໍ່ໄດ້ກວດ(0)						*feverRDT*
15	ເຈົ້າໄດ້ຮັກສາປິ່ນປົວບໍ? ບໍ່ໄດ້ຮັກສາປິ່ນປົວ(0), ການກິນຢາປົວໂຣກຍຸງ(1) (Choloroquine ຫຼື CQ), ການປິ່ນປົວດ້ວຍການປະສົມຢາອາກເຕີມີນິນ (ACT ຫຼື Artemsinin Combination Therapy) (2) *ໄດ້ຮັບການປິ່ນປົວນອກຈາກວິທີທີ່ໄດ້ເວົ້າມາຂ້າງເທິງນີ້ ແຕ່ບໍ່ຮູ້ວ່າແມ່ນວິທີໃດ(3)*						*feverimi*

ຊື່ຂອງຜູ້ໃຫ້ສຳພາດ

English language exit survey form

Page 1

Malaria Repellent Trial Exit Survey December 2009

Interviewer Name		Province		Household	
Date		District		Group	

Participants	What is your relationship to the head of the household? *I am the head of household (1), Spouse of HH (2), Child (3), Parent of HH (4), Sibling of HH (5), Other relation (6), Non relation (7)*	Did you sleep under a bednet every night this month? *Yes (1), No (0)*	If NOT, why not? *Forgot (1), Did not have net (2), Too hot (3), Other (state)*	Did you work in the fields or forest this month? *Yes (1), No (0)*	Did you sleep in the fields or forest this month? *Yes (1), No (0)*	If YES, how many nights did you sleep in the fields or forest this month?	If YES, did you use a bed net? *Yes (1), No (0)*
1							
2							
3							
4							
5							

Participants	What do you do during your work out side of village? *Don't go(0), Mountain rice paddies (1), Lowland rice paddies and gardens (2), Forest product collection or hunting (3), Rubber tree plantation (4), Other (5)*	Did you use the lotion every evening this month? *Yes (1), No (0)*	If NOT, why not? *Forgot (1), Smell (2), Allergy (3), Other (state)*	Did you use the lotion during the day? *Yes (1), No (0)*	If YES, how many days this month?	How much lotion is returned (from 3 x 50ml bottles)? *None (0) to All (15)*
1						
2						
3						
4						
5						

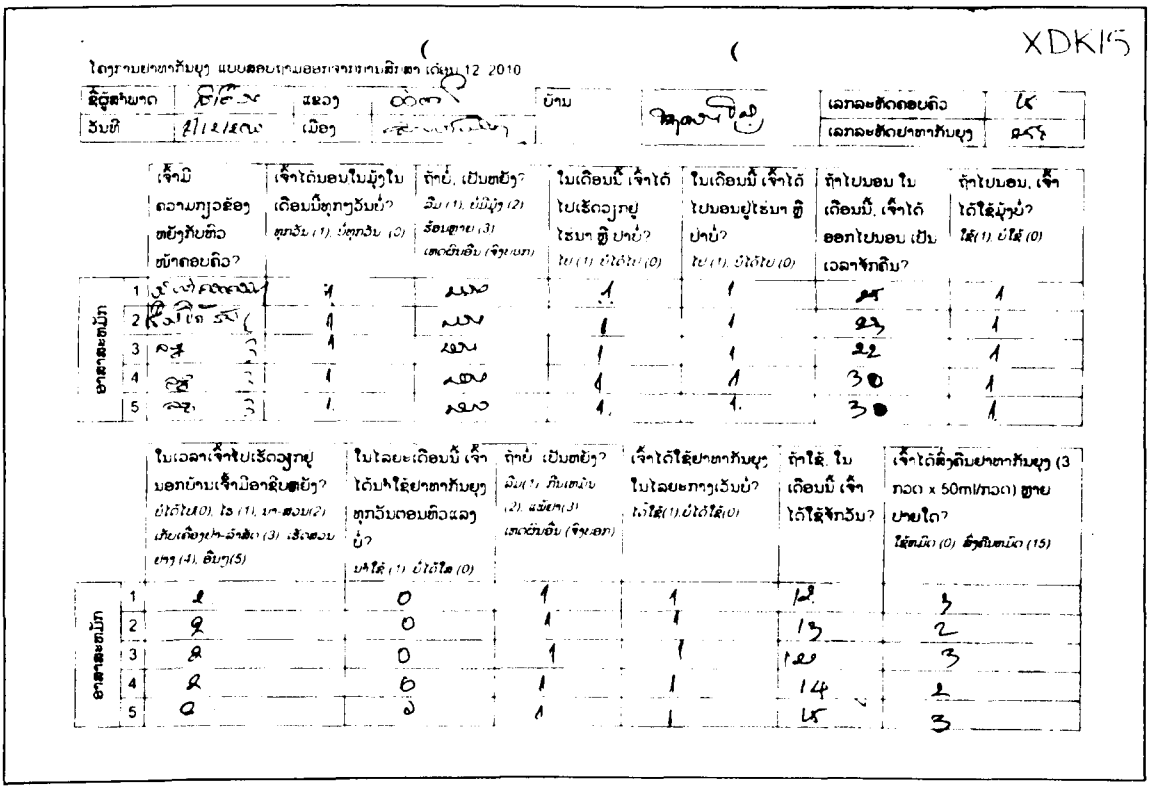
Page 2

Malaria Repellent Trial Exit Survey December 2009

Participants	Did you have a fever this month? *None (0), One (1), More than one (2)*	If you had a fever, did you have an RDT? *Yes (1), No (0)*	What treatment did you receive? *None (0), Co-artem (1), Other (2), Treated but not sure what (3)*	Did you like using the lotion? *Yes (1), No (0)*	If NO, why not? *Bad smell (1), Allergy (2), Headache (3), Does not stop bites (4), Other (state)*	Would you continue using insect repellent if cost was no problem? *Yes (1), No (0)*	Do you think insect repellent is a good way to reduce malaria? *Yes (1), No (0)*
1							
2							
3							
4							
5							

Participants	When or where do you think you get bitten most by mosquitoes?				Did you have malaria this year? *Yes (1), No (0)*	Why do you think you did / did not?	RDT results for *Pf*? *Positive (1), Negative (0)*	RDT results for *Pv*? *Positive (1), Negative (0)*	What ethnic group do you belong to?
	Dry season (1) OR Wet season (2)	*Indoors (3) OR Outdoors (4)*	*Village (5), Ricefield (6) OR Forest (7)*	*Evening (8) OR Night (9)*					
1									
2									
3									
4									
5									

Lao language exit survey form (completed example)



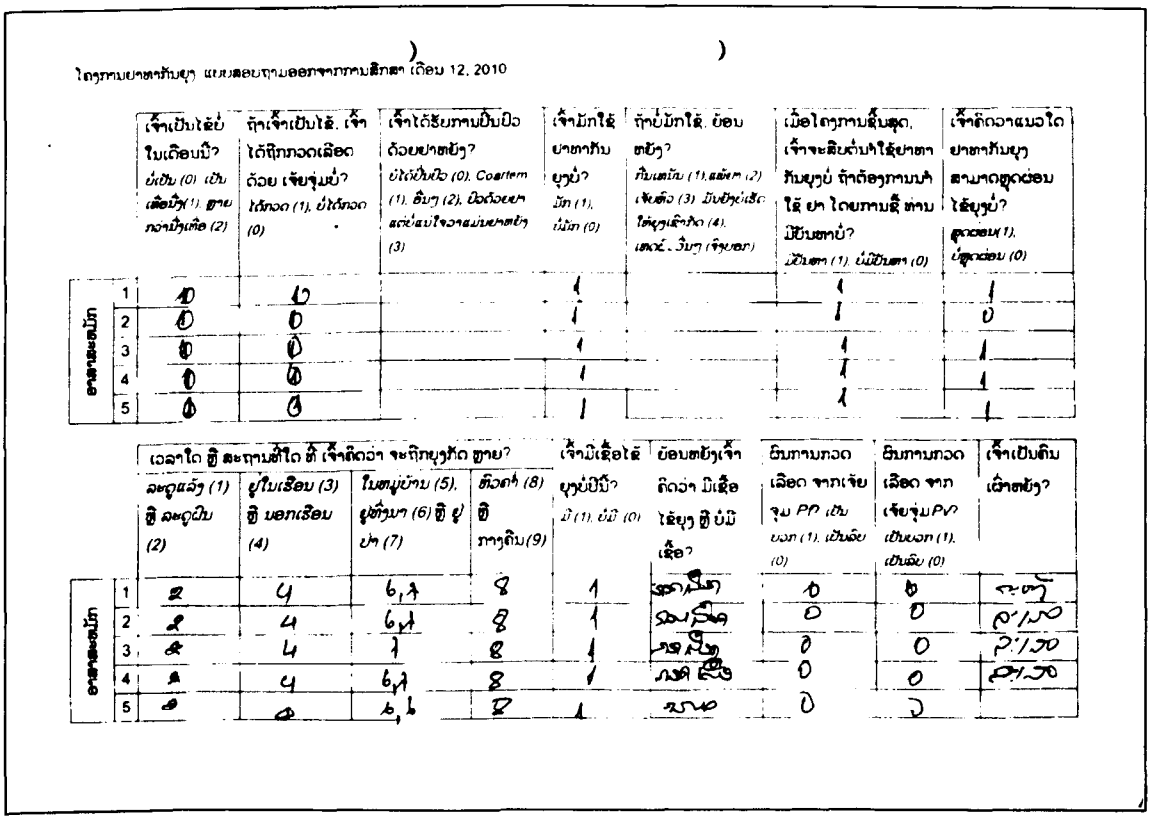
ໂຄງການຢາຫາກັນຍຸງ ແບບສອບຖາມອອກຈາກການສຶກສາ ເດືອນ 12, 2010

XDK15

ຊື່ຜູ້ສຳພາດ		ແຂວງ		ບ້ານ		ເລກລະຫັດຄອບຄົວ	
ວັນທີ	2/12/2010	ເມືອງ				ເລກລະຫັດຢາຫາກັນຍຸງ	

		ເຈົ້າມີຄວາມກັງວົນຫຍັງກ່ຽວກັບຫົວໜ້າຄອບຄົວ?	ເຈົ້າໄດ້ນອນໃນມຸ້ງໃນເດືອນນີ້ທຸກໆວັນບໍ່? *ທຸກວັນ (1), ບໍ່ທຸກວັນ (0)*	ຖ້າບໍ່, ເປັນຫຍັງ? *ລືມ (1), ບໍ່ມີມຸ້ງ (2), ຮ້ອນເກີນ (3), ເຫດຜົນອື່ນ (ຈົ່ງບອກ)*	ໃນເດືອນນີ້ ເຈົ້າໄດ້ໄປເຮັດວຽກຢູ່ໄຮ່ນາ ຫຼື ປ່າບໍ່? *ໄປ (1), ບໍ່ໄດ້ໄປ (0)*	ໃນເດືອນນີ້ ເຈົ້າໄດ້ໄປນອນຢູ່ໄຮ່ນາ ຫຼື ປ່າບໍ່? *ໄປ (1), ບໍ່ໄດ້ໄປ (0)*	ຖ້າໄປນອນ ໃນເດືອນນີ້, ເຈົ້າໄດ້ອອກໄປນອນ ເປັນເວລາຈັກຄືນ?	ຖ້າໄປນອນ, ເຈົ້າໄດ້ໃຊ້ມຸ້ງບໍ່? *ໃຊ້ (1), ບໍ່ໃຊ້ (0)*
ອາສາສະໝັກ	1	ບໍ່	1	ນອນ	1	1	25	1
	2	ບໍ່	1	ນອນ	1	1	23	1
	3	ບໍ່	1	ນອນ	1	1	22	1
	4	ບໍ່	1	ນອນ	1	1	30	1
	5	ບໍ່	1	ນອນ	1	1	30	1

		ໃນເວລາເຈົ້າໄປເຮັດວຽກຢູ່ນອກບ້ານເຈົ້າມີອາຊີບຫຍັງ? *ບໍ່ໄດ້ໄປ(0), ໄຮ (1), ນາ-ສວນ(2), ລ້ຽງເດັກ/ເຝົ້າເຮືອ (3), ເຮັດສວນ ຍາງ (4), ອື່ນໆ(5)*	ໃນໄລຍະເດືອນນີ້ ເຈົ້າໄດ້ນຳໃຊ້ຢາຫາກັນຍຸງ ທຸກວັນຕອນຫົວຄ່ຳ ບໍ? *ນຳໃຊ້ (1), ບໍ່ໄດ້ໃຊ້ (0)*	ຖ້າບໍ່ ເປັນຫຍັງ? *ລືມ(1), ກິນເຫຼົ້າ (2), ເມື່ອຍ(3), ເຫດຜົນອື່ນ (ຈົ່ງບອກ)*	ເຈົ້າໄດ້ໃຊ້ຢາຫາກັນຍຸງ ໃນໄລຍະກາງເວັນບໍ? *ໄດ້ໃຊ້(1),ບໍ່ໄດ້ໃຊ້(0)*	ຖ້າໃຊ້, ໃນເດືອນນີ້ ເຈົ້າໄດ້ໃຊ້ຈັກວັນ?	ເຈົ້າໄດ້ສິ່ງຄືນຢາຫາກັນຍຸງ (3 ກວດ x 50ml/ກວດ) ຫຼາຍປານໃດ? *ໃຊ້ໝົດ (0), ສົ່ງຄືນໝົດ (15)*
ອາສາສະໝັກ	1	ຂ	0	1	1	12	3	
	2	ຊ	0	1	1	13	2	
	3	ຊ	0	1	1	12	3	
	4	ຊ	0	1	1	14	2	
	5	ຊ	0	1	1	15	3	

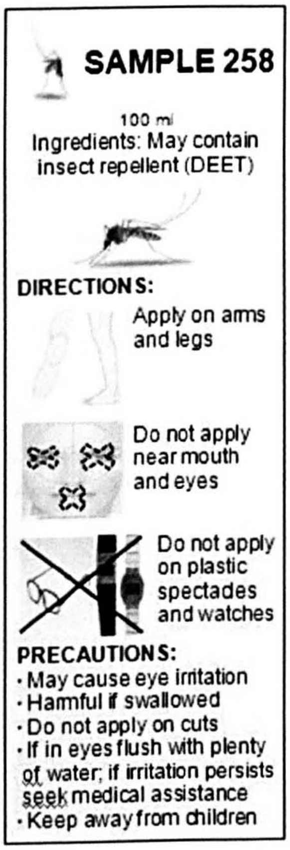


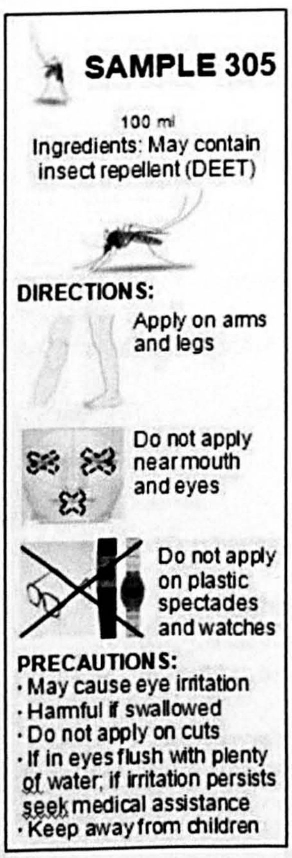
ໂຄງການຢາຫາກັນຍຸງ ແບບສອບຖາມອອກຈາກການສຶກສາ ເດືອນ 12, 2010

		ເຈົ້າເປັນໄຂ້ບໍ່ໃນເດືອນນີ້? *ບໍ່ເປັນ (0), ເປັນເດືອນນີ້(1), ຫຼາຍກວ່ານີ້ເກົ່າ (2)*	ຖ້າເຈົ້າເປັນໄຂ້, ເຈົ້າໄດ້ຖືກກວດເລືອດດ້ວຍ ເຈ້ຍຈຸ່ມບໍ່? *ໄດ້ກວດ (1), ບໍ່ໄດ້ກວດ (0)*	ເຈົ້າໄດ້ຮັບການປິ່ນປົວດ້ວຍຢາຫຍັງ? *ບໍ່ໄດ້ປິ່ນປົວ (0), Coartem (1), ອື່ນໆ (2), ປົວດ້ວຍຢາແຕ່ບ່ແນ່ໃຈວ່າແມ່ນຢາຫຍັງ (3)*	ເຈົ້າມັກໃຊ້ຢາຫາກັນຍຸງບໍ່? *ມັກ (1), ບໍ່ມັກ (0)*	ຖ້າບໍ່ມັກໃຊ້, ຍ້ອນຫຍັງ? *ກິ່ນເໝັນ (1),ແພ້ຢາ (2) ເຈັບຕົ້ວ (3) ມັນຍ້ຽວ(ເຮັດໃຫ້ຍຸງເຂົ້າກັດ (4), ເຫດ ອື່ນໆ (ຈົ່ງບອກ)*	ເມື່ອໂຄງການຊຶ້ນສຸດ, ເຈົ້າຈະສືບຕໍ່ນຳໃຊ້ຢາຫາກັນຍຸງບໍ່ ຖ້າຕ້ອງການນຳໃຊ້ ຢາ ໂດຍການຊື້ ທ່ານມີປັນຫາບໍ? *ມີປັນຫາ (1), ບໍ່ມີປັນຫາ (0)*	ເຈົ້າຄິດວ່າແນວໃດ ຢາຫາກັນຍຸງ ສາມາດຫຼຸດຜ່ອນໄຂ້ຍຸງບໍ່? *ຫຼຸດຜ່ອນ(1), ບໍ່ຫຼຸດຜ່ອນ (0)*
ອາສາສະໝັກ	1	ບ	ບ		1		1	1
	2	ບ	ບ		1		1	0
	3	ບ	ບ		1		1	1
	4	ບ	ບ		1		1	1
	5	ບ	ບ		1		1	1

		ເວລາໃດ ຫຼື ສະຖານທີ່ໃດ ທີ່ ເຈົ້າຄິດວ່າ ຈະຖືກຍຸງກັດ ຫຼາຍ? ລະດູແລ້ງ (1) ຫຼື ລະດູຝົນ (2)	ຢູ່ໃນເຮືອນ (3) ຫຼື ນອກເຮືອນ (4)	ໃນໝູ່ບ້ານ (5), ຢູ່ທົ່ງນາ (6) ຫຼື ຢູ່ປ່າ (7)	ຕົ້ວຕ່ຳ (8) ຫຼື ກາງຄືນ(9)	ເຈົ້າມີເຊື້ອໄຂ້ຍຸງບໍ່ປັດຈຸບັນ? *ມີ (1), ບໍ່ມີ (0)*	ຍ້ອນຫຍັງເຈົ້າຄິດວ່າ ມີເຊື້ອໄຂ້ຍຸງ ຫຼື ບໍ່ມີເຊື້ອ?	ຮັບການກວດເລືອດ ຈາກເຈ້ຍຈຸ່ມ PF ເປັນບວກ (1), ເປັນລົບ (0)	ຮັບການກວດເລືອດ ຈາກເຈ້ຍຈຸ່ມ PV ເປັນບວກ (1), ເປັນລົບ (0)	ເຈົ້າເປັນຄົນເຜົ່າຫຍັງ?
ອາສາສະໝັກ	1	2	4	6,7	8	1	ເຄີຍເປັນ	0	0	ລະຕູ
	2	2	4	6,7	8	1	ເຄີຍເປັນ	0	0	ລ/ເ
	3	2	4	7	8	1	ເຄີຍເປັນ	0	0	ລ/ເ
	4	2	4	6,7	8	1	ເຄີຍເປັນ	0	0	ລ/ເ
	5	2	4	6,7	8	1	ເຄີຍ	0	0	

Bottle Labels





Appendix C: Entomology Survey Data

Table C1. Average mosquito catch by CDC light trap night (CDC, n=301 nights) and man-hour from landing collections (HLC, n=784 hours).

Genus (Subgenus)	Species	Average catch	
		CDC	HLC
Anopheles (Anopheles)	*baezai* Gater, 1933	0.003	0.000
	barbirostris van der Wulp, 1884 / *campestris* Reid, 1962 §	0.003	0.000
	hodgkini Reid, 1962	0.007	0.000
	peditaeniatus (Leicester, 1908)	0.023	0.000
	pursati Laveran, 1902	0.013	0.001
	roperi Reid, 1950	0.007	0.000
	umbrosus (Theobald, 1903)	0.003	0.000
Anopheles (Cellia)	*annularis* van der Wulp, 1884	0.130	0.003
	culicifacies Giles, 1901	0.030	0.001
	dirus Peyton & Harrison, 1979	0.007	0.000
	dravidicus Christophers, 1924	0.007	0.000
	indefinitus (Ludlow, 1904)	0.003	0.000
	jamesii Theobald, 1901	0.040	0.000
	jeyporiensis James, 1902	0.173	0.001
	kochi Dönitz, 1901	0.016	0.000
	maculatus K / sawadwongporni Rattanarithikul & Green, 1987 §	0.146	0.000
	minimus Theobald, 1901	0.053	0.003
	nemophilous Peyton & Ramalingam, 1988	0.033	0.000
	nivipes (Theobald, 1903)	0.027	0.000
	notanandai Rattanarithikul & Green, 1987	0.003	0.000
	pamapanai Büttiker & Beales, 1959	0.003	0.000
	philippinensis Ludlow, 1902	0.010	0.000

	subpictus Grassi, 1899	0.003	0.000
	tessellatus Theobald, 1901	0.010	0.000
	vagus Dönitz, 1902	0.007	0.000
Anopheles	Unidentified	0.143	0.000
Armigeres	Not identified to species	0.907	0.056
Coquilettidia	Not identified to species	0.120	0.001
	alis Theobald, 1903	0.047	0.000
	barraudi Edwards, 1922 / *edwardsi* Barraud, 1923 §	0.090	0.000
	fuscocephala Theobald, 1907	0.010	0.000
	gelidus Theobald, 1901	1.728	0.010
	hutchinsoni Barraud, 1924	0.375	0.000
	perplexus Leicester, 1908	0.259	0.000
Culex (Culex)	*pseudovishnui* Colless, 1957	0.017	0.000
	quinquefasciatus Say, 1823	10.179	0.042
	sitiens Wiedemann, 1828	0.017	0.000
	tritaeniorhynchus Giles, 1901	0.003	0.000
	vishnui Theobald, 1901	2.203	0.001
	whitei Barraud, 1923	14.319	0.284
	whitmorei (Giles, 1904)	0.116	0.001
Culex (Culiciomyia)	*nigropunctatus* Edwards, 1926	0.047	0.000
	foliatus Brug, 1932	0.010	0.000
Culex (Eumelanomyia)	*malayi* (Leicester, 1908)	0.213	0.029
	tenuipalpis Barraud, 1924	3.601	0.089
Culex (Oculeomyia)	*bitaeniorhynchus* Giles, 1901	0.003	0.000
Culex	Unidentified	13.987	0.023
Lutzia	*fuscanus* (Wiedemann, 1820)	0.017	0.000
(Metalutzia)	*halifaxii* (Theobald, 1903)	0.007	0.000

	scanloni Bram, 1967	0.532	0.046
	sinensis Theobald, 1903	0.013	0.000
Mansonia	Not identified to species	0.196	0.000
Mimomyia	Not identified to species	0.007	0.000
Ochleratatus	*niveus* Edwards, 1926	0.023	0.001
Orthopodomyia	Not identified to species	0.017	0.000
Stegomyia	*albopicta* (Skuse, 1895)	0.140	0.027
	Unidentified	0.037	0.068
Topomyia	Not identified to species	0.007	0.000
Tripteroides	Not identified to species	0.276	0.060
Uranotaenia	Not identified to species	0.173	0.000
Total		50.595	0.749

§ Not distinguishable by morphology

Appendix D: Poisson regression STATA outputs

Intention to Treat Analysis

```
. spearman malaria falciparum vivax pca1 pca2 age female net forest, stats(p) pw
(obs=varies)

+-----------------+
| Key             |
|-----------------|
|   Sig. level    |
+-----------------+

             | malaria falcip~m    vivax     pca1     pca2      age   female      net   forest
-------------+------------------------------------------------------------------------------
     malaria |
  falciparum |   0.0000
       vivax |   0.0000   0.0000
        pca1 |   0.0003   0.0013   0.1136
        pca2 |   0.0364   0.0337   0.3255   0.0000
         age |   0.2015   0.3077   0.2740   0.0000   0.1226
      female |   0.0166   0.0179   0.2084   0.0000   0.0001   0.0001
         net |   0.3199   0.4787   0.3363   0.0009   0.0000   0.0000   0.0000
      forest |   0.8415   0.8987   0.2753   0.0109   0.0000   0.0000   0.0000   0.4261


. tsset ppt Date
       panel variable:  ppt (unbalanced)
        time variable:  Date, 27 Jul 09 to 22 Jan 11, but with gaps
                delta:  1 day

. xtmepoisson malaria tmt pca1 pca2 female, || household:, covariance(independent) ||
ppt:, covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -562.65099
Iteration 1:   log likelihood = -520.02699
Iteration 2:   log likelihood = -515.33894

Performing gradient-based optimization:

Iteration 0:   log likelihood = -515.33894
Iteration 1:   log likelihood =   -511.653
Iteration 2:   log likelihood = -511.41733
Iteration 3:   log likelihood = -511.41107
Iteration 4:   log likelihood = -511.41104

Mixed-effects Poisson regression                Number of obs      =     44024

------------------------------------------------------------------------------
                |   No. of       Observations per Group       Integration
 Group Variable |   Groups    Minimum    Average    Maximum      Points
----------------+-------------------------------------------------------------
      household |     1398          2       31.5         40           7
            ppt |     6945          1        6.3          8           7
------------------------------------------------------------------------------

                                                Wald chi2(4)       =     12.80
Log likelihood = -511.41104                     Prob > chi2        =    0.0123

------------------------------------------------------------------------------
     malaria |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |    .961611   .2844174    -0.13   0.895     .5385649    1.716963
        pca1 |    .765376    .076445    -2.68   0.007     .6292999    .9308764
        pca2 |   1.138898   .1483198     1.00   0.318     .8823317    1.470068
      female |   .6237954   .1513094    -1.95   0.052     .3877685    1.003487
       _cons |   .0004593   .0001785   -19.78   0.000     .0002145    .0009837
------------------------------------------------------------------------------

------------------------------------------------------------------------------
```

```
  Random-effects Parameters      Estimate   Std. Err.      [95% Conf. Interval]
-----------------------------------------------------------------------------
household: Identity
                    sd(_cons)    1.707514   .2113262       1.33973    2.176262
-----------------------------------------------------------------------------
ppt: Identity
                    sd(_cons)    .0000111   .4456016             0           .
-----------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =    50.04   Prob > chi2 = 0.0000

Note: LR test is conservative and provided only for reference.

. xtmepoisson malaria tmt pca1 female, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood =  -563.2859
Iteration 1:   log likelihood = -520.54667
Iteration 2:   log likelihood = -515.87283

Performing gradient-based optimization:

Iteration 0:   log likelihood = -515.87283
Iteration 1:   log likelihood = -512.16255
Iteration 2:   log likelihood = -511.90611
Iteration 3:   log likelihood = -511.89979
Iteration 4:   log likelihood = -511.89976

Mixed-effects Poisson regression                Number of obs      =     44024

------------------------------------------------------------------------------
                |  No. of       Observations per Group                Integration
 Group Variable |  Groups    Minimum    Average    Maximum            Points
----------------+-------------------------------------------------------------
      household |    1398          2       31.5         40                 7
            ppt |    6945          1        6.3          8                 7
------------------------------------------------------------------------------

                                                Wald chi2(3)       =     11.72
Log likelihood = -511.89976                     Prob > chi2        =    0.0084

------------------------------------------------------------------------------
     malaria |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   .9582312   .2839289    -0.14   0.886     .5361112    1.712717
        pca1 |   .7559783   .0765574    -2.76   0.006     .6198815    .9219555
      female |   .6232583   .1512416    -1.95   0.051     .3873586     1.00282
       _cons |   .0004562   .0001776   -19.76   0.000     .0002127    .0009783
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters  |   Estimate   Std. Err.      [95% Conf. Interval]
-----------------------------+------------------------------------------------
household: Identity          |
                    sd(_cons) |    1.71624   .2114379      1.348066    2.184965
-----------------------------+------------------------------------------------
ppt: Identity                |
                    sd(_cons) |   .0000103   .4454839             0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =    50.72   Prob > chi2 = 0.0000

Note: LR test is conservative and provided only for reference.

. xtmepoisson falciparum tmt pca1 pca2 female, || household:, covariance(independent) ||
ppt:, covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -454.72452
```

```
Iteration 1:   log likelihood = -417.98409
Iteration 2:   log likelihood = -412.56714

Performing gradient-based optimization:

Iteration 0:   log likelihood = -412.56714
Iteration 1:   log likelihood = -410.06261
Iteration 2:   log likelihood = -409.99176
Iteration 3:   log likelihood = -409.99044
Iteration 4:   log likelihood = -409.99044

Mixed-effects Poisson regression                Number of obs      =     44024

------------------------------------------------------------------------------
                  |  No. of       Observations per Group       Integration
 Group Variable   |  Groups    Minimum    Average    Maximum      Points
------------------+-----------------------------------------------------------
      household   |    1398          2       31.5         40           7
            ppt   |    6945          1        6.3          8           7
------------------------------------------------------------------------------

                                                Wald chi2(4)       =     13.16
Log likelihood = -409.99044                     Prob > chi2        =    0.0105

------------------------------------------------------------------------------
  falciparum |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |  .8673835   .2927589    -0.42   0.673     .4476246     1.68077
        pca1 |  .7688484   .0867979    -2.33   0.020      .616234     .9592588
        pca2 |  1.232207   .1788878     1.44   0.150     .9270627     1.637791
      female |  .5365387   .1490326    -2.24   0.025     .3112903      .924776
       _cons |   .000327   .0001476   -17.77   0.000      .000135     .0007923
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters  |   Estimate   Std. Err.      [95% Conf. Interval]
-----------------------------+------------------------------------------------
household: Identity          |
                  sd(_cons)  |   1.815903   .2383784      1.403955    2.348725
-----------------------------+------------------------------------------------
ppt: Identity                |
                  sd(_cons)  |   6.42e-09   .3770199            0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =    46.31   Prob > chi2 = 0.0000

Note: LR test is conservative and provided only for reference.

. xtmepoisson falciparum tmt pca1 female, || household:, covariance(independent) ||
ppt:, covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -455.87171
Iteration 1:   log likelihood =  -419.0271
Iteration 2:   log likelihood = -413.84429

Performing gradient-based optimization:

Iteration 0:   log likelihood = -413.84429
Iteration 1:   log likelihood = -411.08138
Iteration 2:   log likelihood =  -411.0025
Iteration 3:   log likelihood =  -411.0009
Iteration 4:   log likelihood =  -411.0009

Mixed-effects Poisson regression                Number of obs      =     44024

------------------------------------------------------------------------------
                  |  No. of       Observations per Group       Integration
 Group Variable   |  Groups    Minimum    Average    Maximum      Points
------------------+-----------------------------------------------------------
      household   |    1398          2       31.5         40           7
            ppt   |    6945          1        6.3          8           7
------------------------------------------------------------------------------
```

```
                                                Wald chi2(3)      =     11.18
Log likelihood = -411.0009                      Prob > chi2       =    0.0108

------------------------------------------------------------------------------
  falciparum |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   .8642107   .2915873    -0.43   0.665     .4460891     1.67424
        pcal |   .7559033   .0877263    -2.41   0.016     .6021159    .9489697
      female |   .5363298    .149007    -2.24   0.025     .3111322    .9245255
       _cons |   .0003296   .0001486   -17.78   0.000     .0001362    .0007976
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters |   Estimate   Std. Err.      [95% Conf. Interval]
----------------------------+-------------------------------------------------
household: Identity         |
                  sd(_cons) |   1.822188    .238557      1.409795    2.355216
----------------------------+-------------------------------------------------
ppt: Identity               |
                  sd(_cons) |   6.01e-08   .3764874            0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =    46.61   Prob > chi2 = 0.0000

Note: LR test is conservative and provided only for reference.

. xtmepoisson vivax tmt pcal female, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -232.59288
Iteration 1:   log likelihood = -208.45415
Iteration 2:   log likelihood = -206.21637

Performing gradient-based optimization:

Iteration 0:   log likelihood = -206.21637
Iteration 1:   log likelihood = -204.24192
Iteration 2:   log likelihood = -203.66292
Iteration 3:   log likelihood = -203.65385
Iteration 4:   log likelihood = -203.65384

Mixed-effects Poisson regression                Number of obs      =     44024

------------------------------------------------------------------------------
                |   No. of       Observations per Group                  Integration
 Group Variable |   Groups    Minimum    Average    Maximum                Points
----------------+-------------------------------------------------------------
      household |     1398          2       31.5         40                     7
            ppt |     6945          1        6.3          8                     7
------------------------------------------------------------------------------

                                                Wald chi2(3)      =      3.55
Log likelihood = -203.65384                     Prob > chi2       =    0.3140

------------------------------------------------------------------------------
       vivax |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   .9059417   .4630505    -0.19   0.847     .3326816    2.467014
        pcal |   .7126458   .1315281    -1.84   0.066     .4963333    1.023232
      female |   .8758515   .3483744    -0.33   0.739      .401662    1.909854
       _cons |   .0000485   .0000402   -11.97   0.000     9.53e-06    .0002466
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters |   Estimate   Std. Err.      [95% Conf. Interval]
----------------------------+-------------------------------------------------
household: Identity         |
                  sd(_cons) |   2.244755   .3585104      1.641433    3.069832
----------------------------+-------------------------------------------------
ppt: Identity               |
                  sd(_cons) |   2.52e-06   .4224303            0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =    40.73   Prob > chi2 = 0.0000
```

```
Note: LR test is conservative and provided only for reference.

. xtmepoisson vivax tmt pcal, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -232.69522
Iteration 1:   log likelihood = -208.46453
Iteration 2:   log likelihood = -206.44294

Performing gradient-based optimization:

Iteration 0:   log likelihood = -206.44294
Iteration 1:   log likelihood = -204.75617
Iteration 2:   log likelihood = -203.76174
Iteration 3:   log likelihood = -203.70995
Iteration 4:   log likelihood = -203.70941
Iteration 5:   log likelihood = -203.70941

Mixed-effects Poisson regression                Number of obs      =     44024

------------------------------------------------------------------------------
                     No. of       Observations per Group       Integration
 Group Variable      Groups    Minimum    Average    Maximum      Points
-------------------------------------------------------------------------------
      household        1398          2       31.5         40           7
            ppt        6945          1        6.3          8           7
------------------------------------------------------------------------------

                                                Wald chi2(2)       =      3.43
Log likelihood = -203.70941                     Prob > chi2        =    0.1799

------------------------------------------------------------------------------
       vivax |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   .9076742   .4643015    -0.19   0.850      .333055    2.473683
        pcal |   .7109188   .1313506    -1.85   0.065     .4949377     1.02115
       _cons |   .0000447   .0000357   -12.55   0.000     9.36e-06    .0002138
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters  |   Estimate   Std. Err.     [95% Conf. Interval]
-----------------------------+------------------------------------------------
household: Identity          |
                  sd(_cons)  |   2.249136   .3586687      1.645413    3.074372
-----------------------------+------------------------------------------------
ppt: Identity                |
                  sd(_cons)  |   1.86e-08   .4269282            0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =    41.05   Prob > chi2 = 0.0000

Note: LR test is conservative and provided only for reference.
```

According to Protocol Analysis: 50% Compliance

```
. spearman malaria falciparum vivax pca1 pca2 age female net forest, stats(p) pw
(obs=varies)

+-----------------+
| Key             |
|-----------------|
|   Sig. level    |
+-----------------+

             | malaria falciparum    vivax     pca1     pca2      age   female      net   forest
-------------+---------------------------------------------------------------------------------
     malaria |
  falciparum |   0.0000
       vivax |   0.0000   0.0000
        pca1 |   0.0034   0.0183   0.0630
        pca2 |   0.0001   0.0001   0.2341   0.0000
         age |   0.4559   0.6148   0.5849   0.0000   0.0279
      female |   0.4281   0.5284   0.5299   0.0001   0.0006   0.1917
         net |   0.3144   0.3794   0.5876   0.0000   0.0000   0.0000   0.0000
      forest |   0.5761   0.8121   0.4279   0.0000   0.0000   0.0000   0.0000   0.0008

. tsset ppt Date
       panel variable:  ppt (unbalanced)
        time variable:  Date, 27 Jul 09 to 22 Jan 11, but with gaps
                delta:  1 day

. xtmepoisson malaria tmt pca1 pca2, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -350.31557
Iteration 1:   log likelihood = -323.08596
Iteration 2:   log likelihood = -321.57024

Performing gradient-based optimization:

Iteration 0:   log likelihood = -321.57024
Iteration 1:   log likelihood = -319.26394
Iteration 2:   log likelihood = -319.21788
Iteration 3:   log likelihood = -319.21732
Iteration 4:   log likelihood = -319.21731

Mixed-effects Poisson regression                Number of obs      =     32892

------------------------------------------------------------------------------
                |   No. of       Observations per Group       Integration
 Group Variable |   Groups    Minimum    Average    Maximum      Points
----------------+-------------------------------------------------------------
      household |     1368          1       24.0         40           7
            ppt |     6627          1        5.0          8           7
------------------------------------------------------------------------------

                                                Wald chi2(3)       =      8.54
Log likelihood = -319.21731                     Prob > chi2        =    0.0360

------------------------------------------------------------------------------
     malaria |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   1.134405   .4146221     0.35   0.730     .5541868    2.322096
        pca1 |   .7918374   .0934416    -1.98   0.048     .6283319    .9978907
        pca2 |   1.413918   .2179861     2.25   0.025     1.045185    1.912738
       _cons |    .000236   .0001424   -13.84   0.000     .0000723     .00077
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters  |   Estimate   Std. Err.     [95% Conf. Interval]
-----------------------------+------------------------------------------------
household: Identity          |
                  sd(_cons)  |    1.73299   .2893351      1.249344    2.403866
-----------------------------+------------------------------------------------
ppt: Identity                |
                  sd(_cons)  |   .3244254   1.328297      .0001062    991.2737
```

```
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =    25.54   Prob > chi2 = 0.0000

Note: LR test is conservative and provided only for reference.

. xtmepoisson falciparum tmt pca1 pca2, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -275.09296
Iteration 1:   log likelihood = -253.77508
Iteration 2:   log likelihood = -252.05164

Performing gradient-based optimization:

Iteration 0:   log likelihood = -252.05164
Iteration 1:   log likelihood = -250.80553
Iteration 2:   log likelihood = -250.71789
Iteration 3:   log likelihood = -250.70766
Iteration 4:   log likelihood = -250.70753
Iteration 5:   log likelihood = -250.70753

Mixed-effects Poisson regression                Number of obs      =     32892

------------------------------------------------------------------------------
                |   No. of       Observations per Group       Integration
 Group Variable |   Groups    Minimum    Average    Maximum      Points
----------------+-------------------------------------------------------------
      household |     1368          1       24.0         40           7
            ppt |     6627          1        5.0          8           7
------------------------------------------------------------------------------

                                                Wald chi2(3)       =      9.60
Log likelihood = -250.70753                     Prob > chi2        =    0.0223

------------------------------------------------------------------------------
  falciparum |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   .9474088   .3886486    -0.13   0.895     .4239896    2.116994
        pca1 |   .8058672   .1025238    -1.70   0.090     .6280182    1.034082
        pca2 |   1.594853   .2641839     2.82   0.005     1.152713    2.206583
       _cons |    .000199   .0001177   -14.41   0.000     .0000625     .000634
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters  |   Estimate   Std. Err.      [95% Conf. Interval]
-----------------------------+------------------------------------------------
household: Identity          |
                  sd(_cons) |   1.720907   .3423673      1.165238    2.541561
-----------------------------+------------------------------------------------
ppt: Identity                |
                  sd(_cons) |   1.08e-07   .7711505            0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =    16.38   Prob > chi2 = 0.0003

Note: LR test is conservative and provided only for reference.

. xtmepoisson falciparum tmt pca2, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -277.62989
Iteration 1:   log likelihood = -255.74821
Iteration 2:   log likelihood =  -253.5642

Performing gradient-based optimization:

Iteration 0:   log likelihood =  -253.5642
```

```
Iteration 1:   log likelihood = -252.37354
Iteration 2:   log likelihood = -252.29816
Iteration 3:   log likelihood = -252.25143
Iteration 4:   log likelihood =  -252.2514

Mixed-effects Poisson regression                Number of obs      =      32892

------------------------------------------------------------------------------
                  No. of       Observations per Group       Integration
 Group Variable     Groups    Minimum    Average    Maximum      Points
-------------+----------------------------------------------------------------
      household       1368          1       24.0         40           7
            ppt       6627          1        5.0          8           7
------------------------------------------------------------------------------

                                                Wald chi2(2)       =       7.34
Log likelihood =  -252.2514                     Prob > chi2        =     0.0255

------------------------------------------------------------------------------
  falciparum |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   .959021   .3974457    -0.10   0.920      .425663    2.160679
        pca2 |   1.511952   .2311626     2.70   0.007     1.120464    2.040227
       cons |   .0001823   .0001072   -14.64   0.000     .0000576    .0005773
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters |   Estimate   Std. Err.     [95% Conf. Interval]
----------------------------+-------------------------------------------------
household: Identity         |
                 sd(_cons) |   1.815313   .3213352      1.28315    2.568182
----------------------------+-------------------------------------------------
ppt: Identity               |
                 sd(_cons) |   .0000192   .7613616            0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =    20.43   Prob > chi2 = 0.0000

Note: LR test is conservative and provided only for reference.

. xtmepoisson vivax tmt pca1 pca2, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -128.72024
Iteration 1:   log likelihood = -119.45524
Iteration 2:   log likelihood = -119.10847

Performing gradient-based optimization:

Iteration 0:   log likelihood = -119.10847
Iteration 1:   log likelihood = -118.79147
Iteration 2:   log likelihood = -118.73531
Iteration 3:   log likelihood = -118.72919
Iteration 4:   log likelihood = -118.72791
Iteration 5:   log likelihood = -118.72791

Mixed-effects Poisson regression                Number of obs      =      32892

------------------------------------------------------------------------------
                |  No. of       Observations per Group       Integration
 Group Variable |  Groups    Minimum    Average    Maximum      Points
----------------+-------------------------------------------------------------
      household |    1368          1       24.0         40           7
            ppt |    6627          1        5.0          8           7
------------------------------------------------------------------------------

                                                Wald chi2(3)       =       3.81
Log likelihood = -118.72791                     Prob > chi2        =     0.2823

------------------------------------------------------------------------------
       vivax |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   1.013961   .5901457     0.02   0.981     .3240447    3.172764
```

```
        pca1     .662968   .1499135    -1.82   0.069     .4256129    1.032691
        pca2     1.148737   .3144042     0.51   0.612     .6718166    1.964223
       _cons     .0000824   .0000939    -8.25   0.000     8.83e-06    .0007688
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters |   Estimate   Std. Err.      [95% Conf. Interval]
----------------------------+-------------------------------------------------
household: Identity         |
                  sd(_cons) |   1.704536   .6394128       .817146    3.555597
----------------------------+-------------------------------------------------
ppt: Identity               |
                  sd(_cons) |   4.16e-07   1.505006            0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =     2.67   Prob > chi2 = 0.2627

Note: LR test is conservative and provided only for reference.

. xtmepoisson vivax tmt pca1, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -128.88051
Iteration 1:   log likelihood = -119.62821
Iteration 2:   log likelihood = -119.26836

Performing gradient-based optimization:

Iteration 0:   log likelihood = -119.26836
Iteration 1:   log likelihood =  -118.9432
Iteration 2:   log likelihood = -118.88511
Iteration 3:   log likelihood = -118.85591
Iteration 4:   log likelihood = -118.85317
Iteration 5:   log likelihood = -118.85288
Iteration 6:   log likelihood = -118.85288

Mixed-effects Poisson regression                Number of obs      =     32892

------------------------------------------------------------------------------
                |   No. of       Observations per Group                  Integration
 Group Variable |   Groups    Minimum    Average    Maximum                 Points
----------------+-------------------------------------------------------------
      household |     1368          1       24.0         40                      7
            ppt |     6627          1        5.0          8                      7
------------------------------------------------------------------------------

                                                Wald chi2(2)       =      3.48
Log likelihood = -118.85288                     Prob > chi2        =    0.1753

------------------------------------------------------------------------------
       vivax |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   1.005379   .5870387     0.01   0.993     .3201215    3.157509
        pca1 |   .6522806   .1494446    -1.86   0.062     .4163082    1.022007
       _cons |   .0000782   .0000897    -8.24   0.000     8.25e-06    .0007412
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters |   Estimate   Std. Err.      [95% Conf. Interval]
----------------------------+-------------------------------------------------
household: Identity         |
                  sd(_cons) |   1.739135   .6332697      .8518953    3.550427
----------------------------+-------------------------------------------------
ppt: Identity               |
                  sd(_cons) |   2.03e-07   1.491323            0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =     2.83   Prob > chi2 = 0.2429

Note: LR test is conservative and provided only for reference.
```

According to Protocol Analysis: 75% Compliance

```
. spearman malaria falciparum vivax pca1 pca2 age female net forest, stats(p) pw
(obs=varies)

+-----------------+
| Key             |
|-----------------|
|   Sig. level    |
+-----------------+

             | malaria falcip~m    vivax     pca1     pca2      age   female      net   forest
-------------+------------------------------------------------------------------------------
     malaria |
  falciparum |   0.0000
       vivax |   0.0000   0.9312
        pca1 |   0.0003   0.0031   0.0421
        pca2 |   0.0119   0.0014   0.9762   0.0000
         age |   0.6222   0.5619   0.9482   0.0000   0.5972
      female |   0.3340   0.2022   0.9646   0.0057   0.0002   0.3250
         net |   0.5280   0.6230   0.6925   0.0249   0.0007   0.0000   0.0000
      forest |   0.8401   0.9721   0.7150   0.0000   0.0000   0.0000   0.0006   0.3209

. tsset ppt Date
       panel variable:  ppt (unbalanced)
        time variable:  Date, 27 Jul 09 to 31 Dec 10, but with gaps
                delta:  1 day

. xtmepoisson malaria tmt pca1 pca2, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -183.15296
Iteration 1:   log likelihood = -168.93313
Iteration 2:   log likelihood = -168.53804

Performing gradient-based optimization:

Iteration 0:   log likelihood = -168.53804
Iteration 1:   log likelihood = -168.13258
Iteration 2:   log likelihood =  -168.1114
Iteration 3:   log likelihood = -168.10808
Iteration 4:   log likelihood = -168.10756
Iteration 5:   log likelihood = -168.10753
Iteration 6:   log likelihood = -168.10753

Mixed-effects Poisson regression                Number of obs      =     22037

------------------------------------------------------------------------------
                |   No. of       Observations per Group       Integration
 Group Variable |   Groups    Minimum    Average    Maximum        Points
----------------+-------------------------------------------------------------
      household |     1342          1       16.4         40             7
            ppt |     6147          1        3.6          8             7
------------------------------------------------------------------------------

                                                Wald chi2(3)       =     11.11
Log likelihood = -168.10753                     Prob > chi2        =    0.0111

------------------------------------------------------------------------------
     malaria |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |    1.40835   .6680392     0.72   0.470      .5558402     3.56838
        pca1 |   .5756266   .1125831    -2.82   0.005      .3923374     .8445433
        pca2 |   1.361821    .311694     1.35   0.177      .8695548     2.132767
       _cons |   .0001947   .0001687    -9.86   0.000      .0000356     .0010638
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters  |   Estimate   Std. Err.      [95% Conf. Interval]
-----------------------------+------------------------------------------------
household: Identity          |
                  sd(_cons)  |   1.489954    .528589       .74335    2.986431
------------------------------------------------------------------------------
```

```
ppt: Identity                |
                  sd(_cons)  |   .1114021   5.232334      1.17e-41    1.06e+39
-------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =     4.96   Prob > chi2 = 0.0838

Note: LR test is conservative and provided only for reference.

. xtmepoisson malaria tmt pca1, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -184.26471
Iteration 1:   log likelihood = -170.12771
Iteration 2:   log likelihood = -169.52587

Performing gradient-based optimization:

Iteration 0:   log likelihood = -169.52587
Iteration 1:   log likelihood = -168.99282
Iteration 2:   log likelihood =  -168.9619
Iteration 3:   log likelihood = -168.95867
Iteration 4:   log likelihood = -168.95792  (not concave)
Iteration 5:   log likelihood = -168.95786
Iteration 6:   log likelihood =  -168.9578
Iteration 7:   log likelihood =  -168.9578

Mixed-effects Poisson regression                Number of obs      =     22037

------------------------------------------------------------------------------
                |  No. of       Observations per Group                Integration
 Group Variable |  Groups    Minimum    Average    Maximum             Points
----------------+-------------------------------------------------------------
      household |    1342          1       16.4         40                  7
            ppt |    6147          1        3.6          8                  7
------------------------------------------------------------------------------

                                                Wald chi2(2)       =      9.04
Log likelihood =  -168.9578                     Prob > chi2        =    0.0109

------------------------------------------------------------------------------
     malaria |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   1.360049   .6572281     0.64   0.525      .5274982    3.506614
        pca1 |   .5517356   .1124133    -2.92   0.004      .3700864    .8225436
       _cons |   .0001612   .0001295   -10.87   0.000      .0000334     .000778
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters  |   Estimate   Std. Err.      [95% Conf. Interval]
-----------------------------+------------------------------------------------
household: Identity          |
                  sd(_cons)  |   1.653499   .4813497      .9345677    2.925479
-----------------------------+------------------------------------------------
ppt: Identity                |
                  sd(_cons)  |   .0005069   6.157531            0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =     7.13   Prob > chi2 = 0.0283

Note: LR test is conservative and provided only for reference.

. xtmepoisson falciparum tmt pca1 pca2 female, || household:, covariance(independent) ||
ppt:, covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -115.59597
Iteration 1:   log likelihood = -106.48128
Iteration 2:   log likelihood = -106.40968
```

```
Performing gradient-based optimization:

Iteration 0:   log likelihood = -106.40968
Iteration 1:   log likelihood = -105.75536
Iteration 2:   log likelihood = -105.71027
Iteration 3:   log likelihood = -105.69933
Iteration 4:   log likelihood = -105.69933

Mixed-effects Poisson regression                Number of obs      =     22037

------------------------------------------------------------------------------
                |   No. of       Observations per Group       Integration
 Group Variable |   Groups    Minimum    Average    Maximum      Points
----------------+-------------------------------------------------------------
      household |      1342          1       16.4         40           7
            ppt |      6147          1        3.6          8           7
------------------------------------------------------------------------------

                                                Wald chi2(4)       =     12.26
Log likelihood = -105.69933                     Prob > chi2        =    0.0155

------------------------------------------------------------------------------
  falciparum |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   1.276856   .7775206     0.40   0.688      .387094    4.211795
        pca1 |   .5661971    .134491    -2.39   0.017     .3554499    .9018968
        pca2 |   2.002911   .5406937     2.57   0.010     1.179985    3.399749
      female |   2.053022   1.224236     1.21   0.228     .6379908     6.60652
       _cons |   .0000586   .0000698    -8.17   0.000     5.66e-06    .0006062
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters      Estimate   Std. Err.     [95% Conf. Interval]
-----------------------------+------------------------------------------------
household: Identity          |
                  sd(_cons) |   1.538643   .7506904      .5913494    4.003424
-----------------------------+------------------------------------------------
ppt: Identity                |
                  sd(_cons) |   6.45e-06   .9075495            0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =     2.38   Prob > chi2 = 0.3036

Note: LR test is conservative and provided only for reference.

. xtmepoisson falciparum tmt pca1 pca2, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -116.43884
Iteration 1:   log likelihood = -107.29601
Iteration 2:   log likelihood =  -107.1196

Performing gradient-based optimization:

Iteration 0:   log likelihood =  -107.1196
Iteration 1:   log likelihood = -106.55122
Iteration 2:   log likelihood = -106.50144
Iteration 3:   log likelihood = -106.48953
Iteration 4:   log likelihood = -106.48951
Iteration 5:   log likelihood = -106.48951

Mixed-effects Poisson regression                Number of obs      =     22037

------------------------------------------------------------------------------
                |   No. of       Observations per Group       Integration
 Group Variable |   Groups    Minimum    Average    Maximum      Points
----------------+-------------------------------------------------------------
      household |      1342          1       16.4         40           7
            ppt |      6147          1        3.6          8           7
------------------------------------------------------------------------------

                                                Wald chi2(3)       =     10.80
Log likelihood = -106.48951                     Prob > chi2        =    0.0129
```

```
------------------------------------------------------------------------------
  falciparum |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |    1.25434    .763255     0.37   0.710      .380598    4.133939
        pca1 |   .5696271   .1347696    -2.38   0.017      .3582635   .9056881
        pca2 |   2.003694   .5431626     2.56   0.010      1.177842   3.408596
       _cons |   .0000898   .0001008    -8.30   0.000      9.95e-06   .0008102
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters  |   Estimate   Std. Err.      [95% Conf. Interval]
-----------------------------+------------------------------------------------
household: Identity          |
                  sd(_cons)  |   1.566382   .7366641      .6231328    3.937449
-----------------------------+------------------------------------------------
ppt: Identity                |
                  sd(_cons)  |   4.88e-09   .9408947            0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =     2.58   Prob > chi2 = 0.2755

Note: LR test is conservative and provided only for reference.

. xtmepoisson vivax tmt pca1, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -81.781505
Iteration 1:   log likelihood = -75.825396
Iteration 2:   log likelihood = -75.724844

Performing gradient-based optimization:

Iteration 0:   log likelihood = -75.724844
Iteration 1:   log likelihood = -75.699682
Iteration 2:   log likelihood = -75.690969  (not concave)
Iteration 3:   log likelihood = -75.690339
Iteration 4:   log likelihood =  -75.68865
Iteration 5:   log likelihood = -75.687425
Iteration 6:   log likelihood = -75.686874
Iteration 7:   log likelihood =  -75.68679
Iteration 8:   log likelihood =  -75.68677
Iteration 9:   log likelihood =  -75.68677

Mixed-effects Poisson regression                Number of obs      =     22037

------------------------------------------------------------------------------
                |   No. of       Observations per Group       Integration
 Group Variable |   Groups    Minimum    Average    Maximum      Points
----------------+-------------------------------------------------------------
      household |     1342          1       16.4         40           7
            ppt |     6147          1        3.6          8           7
------------------------------------------------------------------------------

                                                Wald chi2(2)       =      5.61
Log likelihood =  -75.68677                     Prob > chi2        =    0.0604

------------------------------------------------------------------------------
       vivax |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   1.920826   1.358741     0.92   0.356      .4801429    7.68432
        pca1 |    .509808   .1593142    -2.16   0.031       .276317   .9406017
       _cons |   .0001795   .0001211   -12.79   0.000      .0000479   .0006735
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters  |   Estimate   Std. Err.      [95% Conf. Interval]
-----------------------------+------------------------------------------------
household: Identity          |
                  sd(_cons)  |   3.37e-06    2.61205            0           .
-----------------------------+------------------------------------------------
ppt: Identity                |
                  sd(_cons)  |   5.78e-06   5.520397            0           .
```

```
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =      0.00   Prob > chi2 = 1.0000

Note: LR test is conservative and provided only for reference.
```

According to Protocol Analysis: 90% Compliance

```
. spearman malaria falciparum vivax pcal pca2 age female net forest, stats(p) pw
(obs-varies)

+-----------------+
| Key             |
|-----------------|
|   Sig. level    |
+-----------------+

             | malaria falcip~m    vivax     pcal     pca2      age   female      net   forest
-------------+---------------------------------------------------------------------------------
     malaria |
  falciparum |   0.0000
       vivax |   0.0000   0.9323
        pcal |   0.0009   0.0023   0.1385
        pca2 |   0.0166   0.0026   0.9672   0.0000
         age |   0.5639   0.3159   0.7351   0.0000   0.8660
      female |   0.3085   0.2848   0.7728   0.0072   0.0001   0.2961
         net |   0.5622   0.6494   0.7194   0.0356   0.5455   0.0005   0.0000
      forest |   0.5667   0.9337   0.4132   0.0000   0.0086   0.0000   0.0099   0.7544

. tsset ppt Date
       panel variable:  ppt (unbalanced)
        time variable:  Date, 27 Jul 09 to 31 Dec 10, but with gaps
                delta:  1 day

. xtmepoisson malaria tmt pcal pca2, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -167.01787
Iteration 1:   log likelihood = -153.86475
Iteration 2:   log likelihood = -153.38184

Performing gradient-based optimization:

Iteration 0:   log likelihood = -153.38184
Iteration 1:   log likelihood = -152.94907
Iteration 2:   log likelihood = -152.91723
Iteration 3:   log likelihood = -152.91187
Iteration 4:   log likelihood =  -152.9093
Iteration 5:   log likelihood = -152.90894
Iteration 6:   log likelihood = -152.90878
Iteration 7:   log likelihood = -152.90877

Mixed-effects Poisson regression                Number of obs      =     19367

------------------------------------------------------------------------------
                |   No. of       Observations per Group       Integration
 Group Variable |   Groups    Minimum    Average    Maximum      Points
----------------+-------------------------------------------------------------
      household |     1311          1       14.8         40           7
            ppt |     5810          1        3.3          8           7
------------------------------------------------------------------------------

                                                Wald chi2(3)       =      8.67
Log likelihood = -152.90877                     Prob > chi2        =    0.0339

------------------------------------------------------------------------------
     malaria |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   1.489295   .7518178     0.79   0.430     .5537095    4.005711
        pcal |   .6047212   .1247683    -2.44   0.015     .4035823    .9061045
        pca2 |   1.325184   .3288408     1.13   0.257     .8148031    2.155259
       _cons |   .0001777   .0001525   -10.06   0.000      .000033    .0009559
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters  |   Estimate   Std. Err.     [95% Conf. Interval]
-----------------------------+------------------------------------------------
household: Identity          |
                  sd(_cons)  |   1.590682   .5447165        .81301    3.112222
```

```
------------------------------------------------------------------------------
ppt: Identity
                sd(_cons) |   .0001364   3.770785           0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =     5.45   Prob > chi2 = 0.0656

Note: LR test is conservative and provided only for reference.

. xtmepoisson malaria tmt pca1, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -167.85464
Iteration 1:   log likelihood = -154.81764
Iteration 2:   log likelihood = -154.66896

Performing gradient-based optimization:

Iteration 0:   log likelihood = -154.66896
Iteration 1:   log likelihood = -153.56509
Iteration 2:   log likelihood = -153.51694
Iteration 3:   log likelihood = -153.51262
Iteration 4:   log likelihood = -153.51204
Iteration 5:   log likelihood = -153.51174
Iteration 6:   log likelihood = -153.51174

Mixed-effects Poisson regression                Number of obs      =     19367

------------------------------------------------------------------------------
                |   No. of       Observations per Group       Integration
 Group Variable |   Groups    Minimum    Average    Maximum      Points
----------------+-------------------------------------------------------------
      household |     1311          1       14.8         40           7
            ppt |     5810          1        3.3          8           7
------------------------------------------------------------------------------

                                                Wald chi2(2)       =      7.14
Log likelihood = -153.51174                     Prob > chi2        =    0.0281

------------------------------------------------------------------------------
     malaria |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   1.454528    .748689     0.73   0.467     .5303749    3.988971
        pca1 |   .5784401   .1237898    -2.56   0.011     .3802734    .8798748
       _cons |   .0001436   .0001246   -10.20   0.000     .0000262    .0007868
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters  |   Estimate   Std. Err.      [95% Conf. Interval]
-----------------------------+------------------------------------------------
household: Identity          |
                sd(_cons) |   1.744032     .49744      .9971749    3.050266
-----------------------------+------------------------------------------------
ppt: Identity                |
                sd(_cons) |   .0000197   3.059158           0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =     7.75   Prob > chi2 = 0.0208

Note: LR test is conservative and provided only for reference.

. xtmepoisson falciparum tmt pca1 pca2, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -107.12398
Iteration 1:   log likelihood = -98.525473
Iteration 2:   log likelihood = -98.164925
```

```
Performing gradient-based optimization:

Iteration 0:   log likelihood = -98.164925
Iteration 1:   log likelihood =  -97.87533
Iteration 2:   log likelihood = -97.816448
Iteration 3:   log likelihood = -97.814128
Iteration 4:   log likelihood = -97.814087
Iteration 5:   log likelihood = -97.814087

Mixed-effects Poisson regression                Number of obs      =     19367

------------------------------------------------------------------------------
                     No. of       Observations per Group       Integration
 Group Variable     Groups    Minimum    Average    Maximum      Points
---------------+--------------------------------------------------------------
      household       1311          1       14.8         40           7
            ppt       5810          1        3.3          8           7
------------------------------------------------------------------------------

                                                Wald chi2(3)       =      9.96
Log likelihood = -97.814087                     Prob > chi2        =    0.0189

------------------------------------------------------------------------------
  falciparum          IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
---------------+--------------------------------------------------------------
         tmt     1.560051   .9873801     0.70   0.482     .4512321    5.393582
        pca1     .5569636   .1440143    -2.26   0.024       .33553    .9245327
        pca2     1.988774   .5834757     2.34   0.019     1.119067    3.534393
       _cons     .0000927   .0001031    -8.35   0.000     .0000105    .0008198
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters      Estimate   Std. Err.     [95% Conf. Interval]
-----------------------------+------------------------------------------------
household: Identity          |
                  sd(_cons)  |   1.477231    .766884      .5340217    4.086372
-----------------------------+------------------------------------------------
ppt: Identity                |
                  sd(_cons)  |   2.23e-08    .936546             0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =     2.14   Prob > chi2 = 0.3435

Note: LR test is conservative and provided only for reference.

. xtmepoisson vivax tmt pca1, || household:, covariance(independent) || ppt:,
covariance(independent) irr
Note: single-variable random-effects specification in household equation; covariance
structure set to identity
Note: single-variable random-effects specification in ppt equation; covariance structure
set to identity

Refining starting values:

Iteration 0:   log likelihood = -73.857558
Iteration 1:   log likelihood = -68.538169
Iteration 2:   log likelihood =  -68.46656

Performing gradient-based optimization:

Iteration 0:   log likelihood =  -68.46656  (not concave)
Iteration 1:   log likelihood = -68.461834  (backed up)
Iteration 2:   log likelihood = -68.455953
Iteration 3:   log likelihood = -68.447679
Iteration 4:   log likelihood = -68.446131
Iteration 5:   log likelihood = -68.444246
Iteration 6:   log likelihood = -68.443944
Iteration 7:   log likelihood = -68.443634
Iteration 8:   log likelihood = -68.443545
Iteration 9:   log likelihood = -68.443519
Iteration 10:  log likelihood = -68.443518

Mixed-effects Poisson regression                Number of obs      =     19367

------------------------------------------------------------------------------
                |   No. of       Observations per Group       Integration
 Group Variable |   Groups    Minimum    Average    Maximum      Points
----------------+-------------------------------------------------------------
      household |      1311          1       14.8         40           7
```

```
           ppt       5810          1         3.3          8           7
------------------------------------------------------------------------------

                                                  Wald chi2(2)      =      3.14
Log likelihood = -68.443518                       Prob > chi2       =    0.2076

------------------------------------------------------------------------------
       vivax |        IRR   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         tmt |   1.647323   1.203365     0.68   0.494     .3935301    6.895717
        pcal |   .6062807   .1872482    -1.62   0.105     .3309653    1.110619
       _cons |   .0002371   .0001527   -12.96   0.000     .0000671     .000838
------------------------------------------------------------------------------

------------------------------------------------------------------------------
  Random-effects Parameters |   Estimate   Std. Err.      [95% Conf. Interval]
----------------------------+-------------------------------------------------
household: Identity         |
                  sd(_cons) |   .0000487   3.629167            0           .
----------------------------+-------------------------------------------------
ppt: Identity               |
                  sd(_cons) |   .0001641   7.899616            0           .
------------------------------------------------------------------------------
LR test vs. Poisson regression:      chi2(2) =     0.00   Prob > chi2 = 1.0000

Note: LR test is conservative and provided only for reference.
```

Appendix E: Ethnicity data

Ethnic groups in the Lao PDR were previously divided into three broad geographically-based groups, the Lao Loum (lowland Lao), Lao Theung (midland Lao) and Lao Song (Highland Lao). Ethnic groups are now identified more along linguistic lines and the Lao Front for National Construction currently recognises 49 ethnic groups.

Table E1. Ethnicities of participants recruited to the trial according to current categorisations, phonetic spellings according to Messerli *et al.* [198].

Ethno-linguistic family	Ethno-linguistic category	Ethnic group (alternative names)	Total recruited	% of trial population	% of national population
Lao-Tai	Lao	Lao	792	9.9	54.6
	Tai-Thay	Phou Thay, Tai, Lue, Tai Neua, Sek, Nyouan, Yang, Lao	0	0	10.2
Mon-Khmer	Khmuic	Khmou, Phong, Pray, Ksing Moul, Thene, Oe Dou, Kri	0	0	12.0
	Palaungic	Lamet, Bit, Sam Tao	0	0	0.5
	Katuic	Katang (Kasseng)	30	0.4	2.1
		Makong	0	0	2.1
		Tri	0	0	0.05
		Ta Oy	82	1.0	0.6
		Katu	407	5.1	0.4
		Kriang (Ngae)	537	6.7	0.2
		Souay	317	4.0	0.8
		Pacoh	65	0.8	0.03
	Bahnaric-Khmer	Jrou (Laven)	84	1.1	0.9
		Triang	1,094	13.7	0.5
		Ye	439	5.5	0.02
		Brao (Lavae)	804	10.1	0.4
		Halak	920	11.5	0.04
		Oy	422	5.3	0.4
		Cheng	3	<0.1	0.1
		Sadang (Halang)	40	0.5	0.02
		Nyaheun	22	0.3	0.1
		Lavi	432	5.4	0.02
		Khmer	0	0	0.1
	Vietic	Toum, Ngouan, Meuang, Kri, Phong	0	0	0.08
Sino-Tibetan	Tibeto-Burman	Akha, Singsily, Lahu, Sila, Hanyi, Lolo, Ho	0	0	2.9
Hmong-Mien	Hmong	Hmong	0	0	8.0
	Mien	Lu Mien	0	0	0.5
Lao Theung			190	2.4	
No data			1,300	16.3	
Total			7,980	100.0	

Appendix F: Focus Group Discussion Transcripts

Focus Group Discussion #1

Location	Ban Kasom, Samackixay District, Attapeu Province, Lao PDR
Date	20th April 2010
Moderator	Santi Sayarath
Participants	All took part in the trial last year and reported that they did like using the lotion
	A – 62 years, F
	B – 52 years, M
	C – 21 years, F
	D – 20 years, F
	E – 47 years, M
	F – 13 years, M
	G – 25 years, F
Others Present	Vanessa Chen-Hussey – Repellent Trial Manager
	Dr Hongkham Keomanila – CMPE
	Dr Manivone – Samackixay District Hospital Staff

Transcript

Moderator	What do you do in the evening?
A	That's when everyone is eating. Everyone comes back from the fields or the river and eats at home.
B	I make and repair my fishing nets in the evening
G	My family all go to the river – we wash and collect water for the next day.
Moderator	Do you stay in the house when you eat, or outside or underneath?
B	Underneath
E	Yes, underneath
Moderator	What time do people sleep, is it different for different people?
C	I'd say between 9 and 10pm most people will go to bed. But the houses far from the village will go to bed earlier.
E	Men go to catch fish late at night; maybe 11-12pm
D	And the children will catch frogs at the same time

Moderator	Where do you catch fish
E	The river, or sometime we walk up to the lake [hydropower dam on plateau above village]. It's a long way so that means we will sleep overnight in the forest
Moderator	How many times a month will you do that?
E	Maybe three nights a month
Moderator	Does anyone in the village stay up late?
A	These days the children all stay up very late – they watch TV. From 6pm until late
Moderator	Do they sit under the houses?
D	No, most people have their TVs upstairs
C	And the young men stay up late drinking
Moderator	How do you prevent mosquitoes biting?
B	The insecticide bednets
E	Older people don't sleep under nets – they say they are protected by phi [spirits]
A	They don't like to sleep under nets because it is too hot.
G	Also they don't wear shirts to protect them from biting when they work in the fields
C	When we light the fire near the house at night that protects us from mosquitoes.
E	We also light a fire when we sleep in the forest
A	And in the ricefields
D	We take the citronella plants and put on the skin, but it only lasts 2 hours
F	When we go to collect bamboo shoots in the day there are many mosquitoes.

Focus Group Discussion #2

Location	Ban Kasom, Samackixay District, Attapeu Province, Lao PDR
Date	20th April 2010
Moderator	Santi Sayarath
Participants	All took part in the trial last year and reported that they did not like using the lotion. A – 35, F

	B – 16, M
	C – 40, M
	D – 36, F
	E – 36, F
	F – 57, F
Others Present	Vanessa Chen-Hussey – Repellent Trial Manager
	Dr Hongkham Keomanila – CMPE
	Dr Manivone – Samackixay District Hospital Staff

Transcript

Moderator	You all reported that you did not like using the repellent: what were the main problems you found?
F	I liked the smell of the repellent to start with, but I got tired of it and in the end the smell made me feel sick
A	I also didn't like the smell, it gave me headaches
D	One time my daughter used the repellent when she had a cut on her leg it hurt and made the cut bigger.
E	At the beginning I used the repellent and it was fine, but I used often and I also got a headache from the smell, it was very bad
C	I agree with "E" - when I started using the repellent it was really good and I used it every evening before going to sleep. But after 3-5 days I started to get headaches from the smell and stopped using it.
B	I had a rash from using the repellent
Moderator	Do you remember which number you had?
B	I think 258
Moderator	It seems like the smell of the repellent was a problem for most of you – can you explain a bit more? What would be a better fragrance?
E	Actually I think the smell was good, like a perfume or talcum powder. But maybe it was too strong.
A	Maybe a fruit smell would be good, like orange
D	Orange
B	I don't know
C	I always forgot to use the repellent, that's why I didn't use it much
F	The repellent smells like perfume, whatever is the newest brand, that

	will be the best smell.
D	But I liked the smell at first, it was like talcum powder – very good. But after 4 days it smelled bad to me.
F	It's like when you get the bus and there are many people there all using different perfumes and some not using any who smell very bad. Then all together the different smells give you a headache. And like durian – some people like the smell, some don't.
Moderator	Did you notice whether you got bitten by mosquitoes when you were using the repellent?
F	No, it really can protect, 100%
A	When you put it on you get no mosquitoes on your body.
C	It also worked on other insects and animals that take blood from humans, not just mosquitoes.
Moderator	What animals?
C	Leeches - from the forest
Moderator	You all did not use the repellent very much – can you talk about when you did, where and why?
D	In the evening, and sometimes in the day
E	In the forest
C	And the ricefields – because there are many mosquitoes there
A	I used it in the ricefields too, but only in the beginning
E	Yes, after 4 days I didn't like the smell and didn't want to use it anymore.
Moderator	Was the smell the only problem with the repellent?
D	Only smell was a problem The lotion got in my eyes once and made my eye very red and difficult to open the next day.
B	I was allergic to the lotion and it made me tired.
Moderator	How about eating in the evening, was using the lotion a problem then – maybe you didn't apply the lotion until you finished eating?
F	No, that isn't a problem. Just wash your hands before you eat and the smell isn't a problem for eating.
Moderator	You said earlier that the lotion protected you from mosquitoes biting, but you didn't use the repellent much. So is there anything else you do to avoid getting bitten?

F	Cigarettes can protect you in the forest. It's true! If you smoke cigarettes the mosquitoes won't bite you.
C	Some people use the citronella plant.
Moderator	How do you use the citronella?
C	We crush the plant and mix with water and rub on the skin, but it does not kill the mosquitoes and does not last very long.
E	In the village; the cooking fires outside the house can protect from mosquitoes
D	We sometime take tissue and twist it to make a long rope and burn it near to the body, to keep all insects, not just mosquitoes, away.
E	When the rice is growing, we can use the young leaves [before the rice is harvested] the same way – make a rope and burn when we go to the forest.
A	The best way when you go to the forest to get bamboo shoots is to just wear a longer shirt and pants and cover your face.
C	For men when we are working directly in the field, we just need to wear long clothes.
A	In the village many mosquitoes are only in the evening, around 6pm. But when you go to the forest, they bite all the time.
F	But after the water flood, there were many mosquitoes, even in the village and very big mosquitoes.
Moderator	Thank you for your time. Before we finish are there any other things about the repellent you wanted to talk about?
A	I think I would use the repellent if the smell was different – like an orange smell
D	I think a spray would be good, I have seen other people using it and I think it would be easier to use than the cream.

Focus Group Discussion #3

Location	Lao Yao Kao, Samackixay District, Attapeu Province, Lao PDR
Date	21st April 2010
Moderator	Santi Sayarath
Participants	All took part in the trial last year and reported that they did like using the lotion.

A – 53, M

B – 20, M

C – 44, F

D – 15, F

E – 46, F

F – 60, M

Others Present: Vanessa Chen-Hussey – Repellent Trial Manager

Dr Hongkham Keomanila – CMPE

Dr Manivone – Samackixay District Hospital Staff

Transcript

Moderator	Can you tell me what you do in the evenings?
A	I making fishing nets after I have eaten, probably about 8-9pm
Moderator	What time do you sleep?
B	I used to stay up late talking after I put the repellent on and usually didn't sleep until late – around 10pm
C	We prepare the food and eat, usually that would be 5 or 6pm
F	People watch TV until 8pm or even later
D	I always put the repellent on after eating, so probably around 6pm, then I watch TV before I go to sleep.
E	I prepare food for everyone from 4pm and only after eating I put the repellent on.
Moderator	Does everyone in the village sleep at the same time?
C	Most people sleep from 8pm
A	But depending on the day it can be later
Moderator	What groups were sleeping late?
F	Younger people like to sleep late and they like to drink and may be watching TV.
Moderator	Is the TV under the house or inside?
B	Sometimes up and sometime underneath, since the water flood, mostly up now.
Moderator	What is the best way to protect mosquitoes?
E	Sleeping in the bednet
C	I wear the long clothes to cover the body

A	When we go out to forest, the repellent was very good
Moderator	Anything else?
C	Sometimes we use citronella - we mix with water and put on the skin
F	Another way is to make a fire when we go to the forest to protect mosquitoes.
D	When there are lots of mosquitoes in the village, we also make fires here. This is especially in July during the raining season.
Moderator	Where are the fires – inside the house?
C	Near the house, outside or underneath.
A	But the best way is to sleep under a net
B	I always sleep under a net – I am used to it. Even in the fields, I sleep under a net. It is a habit. If I don't have a net I can't sleep.
Moderator	What group like to go to the filed such forest?.
E	Couples and families go to work in the fields together.
C	Single people and young people do not go often – only after they are married. Then they go girls and boys together.
A	I always used a net when I went to the forest, but not lotion. I'm not used to using the lotion, so my habit is only to use the net.
Moderator	Do you think malaria is a problem in this village?
A	In this village, it used to be younger people like children who were sick with malaria: 7-10 years old
E	Now, it is people in the group of 30 years old who get sick. Because they go to work in the forest.
C	We have good medicine now in the village, you can take medicine and get better.
F	But even now in the current situation, children still get malaria.
Moderator	Did you have any problems when you used the lotion?
C	I had a headache from the smell
Moderator	How about when you went to the forest, did you use the lotion?
B	There are many mosquitoes in the forest and they cannot bite if you put on the repellent.
C	I used the repellent in the evening, in the forest and in the village
A	The forest has many mosquitoes even during the day, so I use the repellent when I went to the forest.
D	I like the lotion, it made my skin nice, so I used it all day, every day.

	Although it made my legs hairy
E	I still have some lotion remaining from last year and I use it everyday.
C	Do you have any left – I would buy some.
A	The raining season is coming soon, will the project come this year and give us more repellent or not?
C	I need more lotion, if the project gives the lotion to the health centre I can buy it from there
Moderator	The project is finished now. So this year if you have a fever you should go to the health centre and get your blood tested and medicine.

Focus Group Discussion #4

Location	Lao Yao Kao, Samackixay District, Attapeu Province, Lao PDR
Date	21st April 2010
Moderator	Santi Sayarath
Participants	All took part in the trial last year and reported that they did not like using the lotion. A – 39, M B – 39, F C – 48, F D – 32, M E – 22, F F – 13, M
Others Present	Vanessa Chen-Hussey – Repellent Trial Manager Dr Hongkham Keomanila – CMPE Dr Manivone – Samackixay District Hospital Staff

Transcript

Leader	How often did you use the lotion last year? Did you like it, or did you not like it?
E	I did use it
Leader	Did you like it?
	[no answer]

Leader	Did you use the repellent?
E	I didn't like to use it - it had a bad smell
C	[Referring to young girl beside her] At the beginning she used to use lotion and finished one bottle. After that she felt itchy, had headaches and felt sick from the smell.
B	The smell is not that bad, but when I use the lotion I feel sick – I had headaches and feel itchy. The district staff told me I should stop using the lotion.
F	I did not have any problem with the smell but when I used the lotion I felt itchy
Leader	Did you think the repellent smelt bad from the start
C	No, just from 4-5 days
E	I had allergic reaction from the first time I used it
D	But after two weeks we went to the forest and she used it no problem
E	Yes, I only had allergic reaction the first time I used it. Then two weeks later I used it again and no problems, so I used it afterward no problems
Leader	Did you use it a lot?
E	Yes very often, no problems except at first
Leader	Do you remember which number you had?
E	305 I think
Leader	Did everyone use the repellent when they visited the forest?
F	I go to the forest to collect bamboo shoots with my mum and I don't use the repellent when I go.
A	I'm not afraid of malaria, I've never had it.
Leader	What do you think is the best way to prevent mosquito bites?
A	Sleeping under a net
D	And it should have insecticide
Leader	Is there anything else

F	The repellent
Leader	But you didn't like the smell
C	If it had a different smell, I would use it.
Leader	What kind of smell
C	Orange
A	The best way is to sleep under the bednet
B	You should wear the long clothes that cover the body
C	Sometimes mosquitoes can bite even if you wear a long shirt.
Leader	Thank you all very much