BMJ

RESEARCH

Plant based insect repellent and insecticide treated bed nets to protect against malaria in areas of early evening biting vectors: double blind randomised placebo controlled clinical trial in the Bolivian Amazon

N Hill, principal science officer,[1] A Lenglet, research fellow,[1] A M Arnéz, senior clinical scientist,[2] I Carneiro, lecturer[1]

[1]Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT

[2]National Bureau of Malaria Control, Ministry of Health, La Paz, Bolivia

Correspondence to: N Hill
nigel.hill@lshtm.ac.uk

doi:10.1136/bmj.39356.574641.55

ABSTRACT

Objective To determine the effectiveness in reducing malaria of combining an insect repellent with insecticide treated bed nets compared with the nets alone in an area where vector mosquitoes feed in the early evening.

Design A double blind, placebo controlled cluster-randomised clinical study.

Setting Rural villages and peri-urban districts in the Bolivian Amazon.

Participants 4008 individuals in 860 households.

Interventions All individuals slept under treated nets; one group also used a plant based insect repellent each evening, a second group used placebo.

Main outcome measure Episodes of *Plasmodium falciparum* or *P vivax* malaria confirmed by rapid diagnostic test or blood slide, respectively.

Results We analysed 15 174 person months at risk and found a highly significant 80% reduction in episodes of *P vivax* in the group that used treated nets and repellent (incidence rate ratio 0.20, 95% confidence interval 0.11 to 0.38, P<0.001). Numbers of *P falciparum* cases during the study were small and, after adjustment for age, an 82% protective effect was observed, although this was not significant (0.18, 0.02 to 1.40, P=0.10). Reported episodes of fever with any cause were reduced by 58% in the group that used repellent (0.42, 0.31 to 0.56, P<0.001).

Conclusions Insect repellents can provide protection against malaria. In areas where vectors feed in the early evening, effectiveness of treated nets can be significantly increased by using repellent between dusk and bedtime. This has important implications in malaria vector control programmes outside Africa and shows that the combined use of treated nets and insect repellents, as advocated for most tourists travelling to high risk areas, is fully justified.

Registration NCT 00144716.

INTRODUCTION

Bed nets impregnated with insecticide are highly effective at reducing morbidity and mortality from malaria.[1] Most successful reports have been from sub-Saharan Africa, where the most important vector species, *Anopheles gambiae*, feeds indoors overnight.[2] Malaria vectors in other parts of the world, however, are less readily controlled by treated bed nets, particularly those species that prefer to feed outdoors or those that feed in the early evening.[2 3] In areas of nocturnal African vectors, researchers have expressed concern that widespread use of treated nets may bring forward feeding behaviour.[4] As sporozoite positive females feed earlier than other mosquitoes,[5] this might also increase the risk of transmission.

Malaria is the most serious parasitic disease in humans, and improvements in prevention are a global priority for those living in or travelling to endemic areas.[6] In the absence of a reliable vaccine and with emerging drug resistance, methods of personal protection are increasingly important for travellers visiting high risk areas. Each year around 2000 people return to the UK with malaria, and in 2003, 16 died.[7] Travellers to tropical countries commonly use insect repellents applied to the skin to protect against biting insects, and this, along with treated bed nets, is recommended by most general practitioners, travel clinics, and travellers' health guides.[8-10] Despite their widespread acceptance and use,[11] insect repellents applied to the skin have not been shown to protect against disease under normal conditions, although when the insecticide permethrin was combined with a repellent in a soap formulation and left to dry on the skin, it offered protection from malaria.[12]

About 36% of the population of the Americas live in areas with a risk of malaria, which includes around 293 million people in 21 endemic countries.[13] Of the 1.14 million cases of malaria reported in the Americas during 2000, 87% were recorded in the Amazonian subregion of South America.[13] The primary malaria vector in the Amazon, *A darlingi*, has a peak biting activity between 8 pm and 10 pm, and more than 80% of feeding occurs before most local people go to bed, where they can be protected by a treated bed net.[14]

Given the early evening feeding activity of the local vector, treated nets will probably need to be supplemented in the few hours just after dusk by some other

control measure to obtain a high level of control. Field evaluations of several plant-based insect repellents and a N,N-diethyl-*m*-toluamide (DEET) standard in this region found that one particular substance, *Eucalyptus maculata citriodon*, provided a high degree of protection (>98%) against *A darlingi* for up to four hours.[3] We selected a plant based repellent as we consider a natural product has the potential for local production, making it a more readily available, cheaper, and thus a more sustainable option for potential large scale use.

We evaluated the clinical benefit of the combined use of insect repellent and treated bed nets in reducing malaria in an area of evening biting vectors.

METHODS

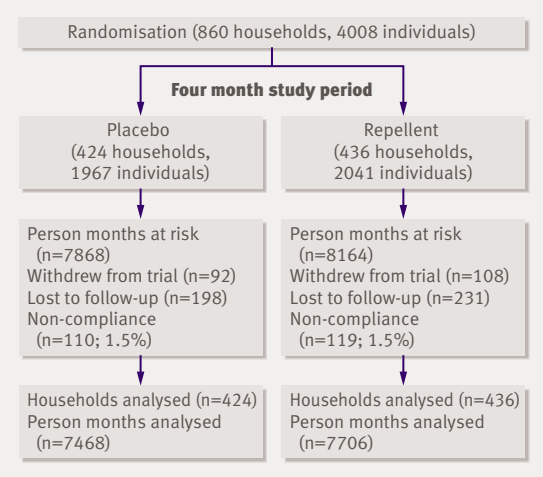
Recruitment

The study was carried out between March and September 2003 in all the rural communities of Vaca Diez and Pando Provinces, Department of Beni, in the Bolivian Amazon Region, plus the outer 10% of the peri-urban districts of the two major towns in the area, Riberalta and Guayaramerin. We recruited up to 20% of households in any one location, and each study house was located a minimum of 25 metres from any other in the study to avoid any effect of diversion of insects from treatment to placebo homes. Researchers collected baseline data (age, sex, occupation) for each participant and obtained written informed consent from each individual or carer of those aged under 18. Participants were allowed to withdraw at any point in the study.

Routine data collected at the health centres reported an annual parasite index for *P falciparum* of 100/1000 population. We calculated the required sample size needed to detect a 30% reduction in the primary outcome of *P falciparum* incidence, assuming a baseline prevalence of 0.1 episodes a year with 90% power to detect the effect at the 95% significance level. We used the methods of Hayes and Bennett[15] for cluster randomised trials with an inter-cluster correlation coefficient of 0.25 and estimated that we needed to recruit and follow-up 408 households in each arm with an average of five individuals per household for the full six month transmission season.

Intervention

Field staff followed the strict inclusion criteria to randomise participants at the household level following a basic sequential alternate A/B/A/B regimen. Field staff and study participants were blind to the group allocation. After we recorded baseline parameters, all participants received a freshly impregnated treated bed net (25 mg/m^2 deltamethrin) plus either the insect repellent (*Eucalyptus maculata citriodon*) with a *p*-menthane 3,8 diol (PMD) concentration of 30% (MASTA, UK) for the treatment group or 0.1% clove oil for the placebo group. Treated nets were also provided to participants in households not enrolled in the study to reduce risks of short range diversion of mosquitoes. Treatment and placebo lotions looked and felt the same, were in identical bottles marked A or B, and had a similar alcohol base formulation and strong natural plant fragrance, but laboratory trials have previously shown that clove oil is ineffective at repelling mosquitoes (N Hill, personal communication, 2003). Individuals were shown how to apply lotion to exposed legs, arms, and neck using a premeasured volume of 10 ml in the bottle cap. Participants applied lotion at dusk each evening. Compliance was monitored by questionnaire and verified by local field staff recording amounts used at monthly follow-up visits and through unannounced evening "sniff checks." To enable a per protocol analysis of efficacy, we considered any individual who reported that they had not used repellent on any three nights (10%) each month or who had more than 30 ml (10%) lotion left as non-compliant and excluded them from analysis for that month.



Study flow of trial

Table 1 | Baseline characteristics of participants and incidence of malaria data by treatment group. Figures are numbers (percentages) unless stated otherwise

	Placebo	Repellent
Households enrolled	424	436
Household television ownership	81 (19)	107 (24)
Individuals enrolled	1967	2041
Mean (SD) age (years)	29.7 (16.9)	29.9 (16.8)
Female	878 (46.3)	934 (47.2)
Positive for *P falciparum*	1 (0.05)	6 (0.3)
Non-compliant person months excluded from analysis	110 (1.5)	119 (1.5)
Person months at risk analysed	7468	7706

Assessments

We recorded malaria infection (with or without fever) at baseline early in the malaria season in March and at active monthly follow-up visits between April and July using *P falciparum* specific rapid diagnostic test (Paracheck dip stick, Orchid PVT, India). As a secondary outcome, the local health district clinic passively detected *P vivax* episodes by microscopic blood slide examination, which was subsequently validated at the central regional health district malaria laboratory in

Riberalta or Guayaramerin. All patients with positive results were referred to the local health centre for prompt treatment: chloroquine and primaquine for *P vivax* or artesunate and mefloquine for *P falciparum*. At each visit researchers asked about and recorded any adverse events.

Statistical analysis

To assess the efficacy of the intervention the analysis included all individuals randomised, but only for the period of time that they were compliant with the intervention. We used Stata 8 with a Poisson regression model to account for the distribution of incidence rates. As the intervention was allocated at the household level, and individual risk within the same household is probably similar because of exposure to other factors, we adjusted for this non-independence of individuals from the same household (intracluster correlation). As there were few episodes of *P falciparum*, we accounted for the intracluster correlation by using robust cluster methods.[16] For *P vivax* and general fever reports, we accounted for the intracluster correlation by using random effects (generalised estimating equation) methods. Because of the potential for relapses, all participants with an episode of *P vivax* were censored at that point and we excluded subsequent episodes and person time of follow-up from the analysis of *P vivax* incidence. We adjusted analyses for age as an a priori covariate because of the effect of age acquired immunity on malaria infection.

RESULTS

The figure shows details of the numbers randomised and the flow through the study. There were no significant differences in most household characteristics (number of household members, roof material, water source, heating source, or possession of electricity, fridge, and radio) between the two groups (data not shown), but households allocated to the repellent group were slightly more likely to own a television than those allocated to the placebo group (P=0.056) (table 1). There were also no significant differences in age or sex between the groups but at baseline more participants in the repellent group were positive for *P falciparum* (P=0.065) (table 1). No adverse events were reported.

As compliance was high for this type of study, with just 1.5% person months excluded in each group, the results of our per protocol analysis would be similar to an intention to treat analysis. The number of *P falciparum* episodes detected was low, and all episodes in the placebo group were in children (age 10-14), while the single episode in the repellent group was in an adult (56 years old) (table 2). Univariate regression analysis suggested an effect of borderline significance, with an 84% reduction in incidence of *P falciparum* in the repellent group (P=0.091). The univariate effect of age group, however, was highly significant with a 95% reduction in incidence in adults (≥15 years) compared with children aged 10-14 (P=0.005). After we accounted for age, the effect of the repellent on incidence of *P falciparum* remained but was even less significant (incidence rate ratio 0.18, 95% confidence interval 0.02 to 1.40, P=0.102).

Analysis of first episodes of *P vivax* showed a reduction in those who used repellent, and again there was a significant influence of age, with a 53% lower incidence in adults compared with children (P=0.002). Even after adjustment for the effect of age, the repellent provided 80% protection (0.20, 0.11 to 0.38, P<0.001).

Similarly, for the analysis of all episodes of fever (reported fever in the past month) there was a 59% reduction in the group that used repellent (P<0.001), a 42% lower incidence of fevers in adults compared with children (P<0.001), and a borderline 56% higher incidence of fevers (P=0.061) in those living in larger households (six or more people) compared with those in smaller households (fewer than six). After adjustment for these factors, there was 58% lower incidence of reported fevers in the repellent compared with the placebo group (0.42, 0.31 to 0.56, P<0.001).

Table 2 | Age specific incidence per 1000 person years (episodes/person months at risk) of different outcomes by treatment group

	Age 10-14 years		Age ≥15 years	
	Repellent	Placebo	Repellent	Placebo
P falciparum	0.0 (0/1641)	40.0 (6/1801)	2.0 (1/6065)	0.0 (0/5667)
P vivax	36.8 (5/1629)	185.7 (27/1745)	17.9 (9/6044)	83.7 (39/5591)
All cause fever	197.4 (27/1641)	719.6 (108/1801)	170.2 (86/6065)	343.0 (162/5667)

DISCUSSION

This randomised controlled trial shows that insect repellent applied to the skin has a significant epidemiological impact on the incidence of malaria. This difference was detected in people who were also sleeping under insecticide treated nets, highlighting the value of additional methods of protection in areas where malaria transmission occurs mainly in the early evening before treated nets are used.

The large effect of the use of repellent on the incidence of *P falciparum* and *P vivax* suggests that most malaria transmission occurs in the early evening, before people are protected by sleeping under a treated bed net. Our results on the effect of the repellent on the incidence of *P falciparum*, however, probably reflect insufficient statistical power because of the overall low incidence of falciparum cases during the study. This might have been because of an unexpected round of outdoor fogging with lambdacyhalothrin by some health districts for a few days mid-way through the trial, which probably temporarily reduced the numbers of local adult mosquitoes, or simply a fluctuation in annual incidence, which is common in South America because of the influence of environmental conditions such as El Niño.

We found clear evidence to support the use of a combination of insect repellent and treated bed nets as personal protection against malaria. This is particularly important to the growing number of

WHAT IS ALREADY KNOWN ON THIS TOPIC

Insecticide treated bed nets are a highly effective means of reducing morbidity and mortality from malaria in Africa, where local vectors bite indoors late at night

Insect repellents can reduce mosquito bites but protection against insect-borne disease is not clear

WHAT THIS STUDY ADDS

Treated bed nets should not be used as the only means of preventing malaria in areas where vectors feed mainly in the evening

Use of an insect repellent can significantly reduce the risk of malaria

The combined use of a repellent and a treated bed net should be advocated to those travelling to malaria risk areas

tourists and business travellers, who have no immunity to malaria. Health professionals and specialist travel health organisations should strongly advocate such combined measures in high risk areas, particularly with early evening or outdoor feeding vectors.

Having established that insect repellents can provide important clinical protection against malaria, we consider their potential use against other insect-borne diseases should be investigated. Ideal targets could include arboviral infections, such as dengue and West Nile fever, transmitted by daytime and outdoor biting culicine mosquito vectors, and leishmaniasis carried by sandflies.

We thank Melissa Rendler-Garcia, Nicola Morgan, and Sharon Slater of Population Services International (PSI), Washington, USA, for their support in Bolivia and assistance in securing funding from PSI for an earlier pilot study. The study would not have been possible without the support and secondment of field staff from the local health districts of Riberalta and Guayaramerin, Bolivia.

Contributors: NH devised the study, was responsible for the protocol development, obtained funding, wrote the first and final drafts of the manuscript, and is guarantor. AL assisted in development of the protocol, acted as study coordinator in Bolivia, trained local field staff, initiated the practical phase of the project, and assisted in production of the manuscript. AMA was field supervisor, undertook daily monitoring of the project in Bolivia, and commented on the manuscript. IC assisted in the study protocol, particularly the analytical methods, study questionnaires, and database design, undertook statistical analysis of results, and contributed to the manuscript.

Funding: Gates Malaria Partnership grant from LSHTM, whose committee reviewed the protocol before the award. Medical Advisory Service for Travellers Abroad (MASTA) provided bulk supplies of the insect repellent at cost price.
Competing interests: NH has received minor funding from numerous manufacturers and suppliers of insect repellents in Europe and the US for laboratory evaluation of their products, and from national consumer groups to compare efficacy of repellents on the European market.
Ethical approval: London School of Hygiene and Tropical Medicine (University of London) ethics committee and Ministerio de Salud y Previsión Social, Bolivia.
Provenance and peer review: Not commissioned; externally peer reviewed.

1 Lengeler C. Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database Syst Rev* 2004;(2):CD000363.
2 Pates H, Curtis C. Mosquito behavior and vector control. *Annu Rev Entomol* 2005;50:53-70.
3 Moore SJ, Lenglet A, Hill N. Field evaluation of three plant-based insect repellents against malaria vectors in Vaca Diez Province, the Bolivian Amazon. *J Am Mosq Control Assoc* 2002;18:107-10.
4 Knoles BGJ, Takken W. The wide-scale use of impregnated bednets for malaria control in Africa: impact on mosquitoes. *Proc Exp Appl Entomol* 1998;8:15-20.
5 Maxwell CA, Wakibara J, Tho S, Curtis CF. Malaria-infective biting at different hours of the night. *Med Vet Entomol* 1998;12:325-7.
6 Greenwood BM, Bojang K, Whitty CJM, Targett GAT. Malaria. *Lancet* 2005;365:1487-98.
7 Health Protection Agency. Malaria imported into the UK, 2003: implications for those advising travellers. www.hpa.org.uk/cdr/archives/2004/cdr3504.pdf.
8 Bradley DJ, Bannister B. Guidelines for malaria prevention in travellers from the United Kingdom for 2003. *Commun Dis Public Health* 2003;6:180-99.
9 NHS Direct. Malaria. www.nhsdirect.nhs.uk/articles/article.aspx?articleId=462.
10 Goodyer LI. Bite avoidance. In: Goodyer LI, ed. *Travel medicine for health professionals*. London: Pharmaceutical Press, 2004:139-71.
11 Fradin MS, Day JF. Comparative efficacy of insect repellents against mosquito bites. *N Engl J Med* 2002;347:13-8.
12 Rowland M. DEET mosquito repellent provides personal protection against malaria: a household randomized trial in an Afghan refugee camp in Pakistan. *Trop Med Int Hlth* 2004;9:335-42.
13 Pan American Health Organization. Basic indicators: health situation in the Americas. www.paho.org/english/dd/ais/BI-brochure-2004.pdf.
14 Harris AF, Matias-Arnéz A, Hill N. Biting time of Anopheles darlingi in the Bolivian Amazon and implication for the control of malaria. *Trans R Soc Trop Med Hyg* 2006;100:45-7.
15 Hayes RJ, Bennett S. Simple sample size calculation for cluster-randomized trials. *Int J Epidemiol* 1999;28:319-26.
16 Horton NJ, Lipsitz SR. Review of software to fit generalized estimating equation regression models. *Am Stat* 1999;53:160-9.

Accepted: 31 August 2007