# Efficacy of topical mosquito repellent (picaridin) plus long-lasting insecticidal nets versus long-lasting insecticidal nets alone for control of malaria: a cluster randomised controlled trial

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## Summary

**Background** Although effective topical repellents provide personal protection against malaria, whether mass use of topical repellents in addition to long-lasting insecticidal nets can contribute to a further decline of malaria is not known, particularly in areas where outdoor transmission occurs. We aimed to assess the epidemiological efficacy of a highly effective topical repellent in addition to long-lasting insecticidal nets in reducing malaria prevalence in this setting.

**Methods** A cluster randomised controlled trial was done in the 117 most endemic villages in Ratanakiri province, Cambodia, to assess the efficacy of topical repellents in addition to long-lasting insecticidal nets in controlling malaria in a low-endemic setting. We did a pre-trial assessment of village accessibility and excluded four villages because of their inaccessibility during the rainy season. Another 25 villages were grouped because of their proximity to each other, resulting in 98 study clusters (comprising either a single village or multiple neighbouring villages). Clusters were randomly assigned (1:1) to either a control (long-lasting insecticidal nets) or intervention (long-lasting insecticidal nets plus topical repellent) study group after a restricted randomisation. All clusters received one long-lasting insecticidal net per individual, whereas those in the intervention group also received safe and effective topical repellents (picaridin KBR3023, SC Johnson, Racine, WI, USA), along with instruction and promotion of its daily use. Cross-sectional surveys of 65 randomly selected individuals per cluster were done at the beginning and end of the malaria transmission season in 2012 and 2013. The primary outcome was *Plasmodium* species-specific prevalence in participants obtained by real-time PCR, assessed in the intention-to-treat population. Complete safety analysis data will be published seperately; any ad-hoc adverse events are reported here. This trial is registered with ClinicalTrials.gov, number NCT01663831.

**Findings** Of the 98 clusters that villages were split into, 49 were assigned to the control group and 49 were assigned to the intervention group. Despite having a successful distribution system, the daily use of repellents was suboptimum. No post-intervention differences in PCR plasmodium prevalence were observed between study groups in 2012 (4·91% in the control group *vs* 4·86% in the intervention group; adjusted odds ratio [aOR] 1·01 [95% CI 0·60–1·70]; p=0·975) or in 2013 (2·96% in the control group *vs* 3·85% in the intervention group; aOR 1·31 [0·81–2·11]; p=0·266). Similar results were obtained according to *Plasmodium* species (1·33% of participants in the intervention group *vs* 1·10% in the intervention group were infected with *Plasmodium falciparum*; aOR 0·83 [0·44–1·56]; p=0·561; and 1·85% in the control group *vs* 2·67% in the intervention group were infected with *Plasmodium vivax*; aOR 1·51 [0·88–2·57]; p=0·133). 41 adverse event notifications from nine villages were received, of which 33 were classified as adverse reactions (11 of these 33 were cases of repellent abuse through oral ingestion, either accidental or not). All participants with adverse reactions fully recovered and 17 were advised to permanently stop using the repellent.

**Interpretation** Mass distribution of highly effective topical repellents in resource-sufficient conditions did not contribute to a further decline in malaria endemicity in a pre-elimination setting in the Greater Mekong subregion. Daily compliance and appropriate use of the repellents remains the main obstacle.

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## Introduction

During the past decade, the scaling up of long-lasting insecticidal nets and, to a lesser extent, indoor residual spraying has contributed to a worldwide decline in the malaria burden.[1] These highly effective vector control tools target mosquitoes resting or feeding indoors at night. Impregnated bednets inhibit blood feeding and induce a mass killing effect on vector populations assuring community protection against malaria.[2] Nonetheless, some malaria vectors show early and

## Research in context

### Evidence before this study

We searched PubMed and Web of Science, with no date or language restrictions, for studies on malaria vector control using topical repellents up to December, 2015, with the terms "anopheles", "malaria", "topical repellent", "vector control", "outdoor biting", and combinations thereof. We found that highly effective vector control measures such as impregnated bednets and indoor residual spraying contributed substantially to a worldwide decline in malaria morbidity and mortality. These control measures target mainly the indoor biting vector population. Persisting transmission because of early and outdoor mosquito biting behaviour hampered further reduction in malaria infections, particularly in low-endemic settings. Topical repellents provided personal protection against indoor and outdoor biting vectors. To what extent mass use of a topical repellent in addition to bednet usage can reduce malaria transmission at the community level remains unclear. A recent meta-analysis based on a small number of studies, but using different study designs, concluded that topical repellents provide individual protection against mosquitoes, but were unlikely to provide effective protection against malaria.

### Added value of this study

We present the results of a large cluster randomised trial designed to assess the efficacy of mass use of topical repellent in addition to insecticide impregnated bednets at the community level. No post-intervention differences for *Plasmodium falciparum* or *Plasmodium vivax* malaria between treatment groups was found. Daily compliance and appropriate use of the repellents, achieved under optimum trial conditions with sufficient resources to promote and distribute the repellent product, remain the main obstacles. Mass distribution of highly effective topical repellents in addition to impregnated bednets did not contribute to a further decrease in malaria endemicity in a pre-elimination setting in Cambodia.

### Implications of all the available evidence

Effective topical repellents, properly applied, provide personal protection against mosquitoes, including malaria vectors. To what extent they can contribute to community-level protection against malaria infection remains unclear. Our study is unique in that it provides a randomised, large-scale, cluster-based design, which allows for the assessment of the efficacy of a topical repellent at the community level. Mass distribution of a topical repellent does not contribute to a further decline in malaria infections at the community level in a low-endemic setting. Therefore, the search for innovative vector control strategies able to halt persisting outdoor malaria transmission, remains ongoing.

outdoor biting behaviour,[3] causing community protection to remain incomplete as a proportion of the vector population is not exposed to the insecticides.[3] To tackle this remaining or residual transmission, additional vector control tools are required, particularly in settings where malaria control programmes move towards elimination.[4]

Topical repellents possess the potential to target residual transmission. Entomological evidence has shown that repellents provide personal protection against malaria.[5,6] However, the efficacy of large-scale coverage of repellents on community protection against malaria remains unverified. Previous studies have assessed the efficacy of topical repellents against malaria infection using individual randomised designs,[7] household randomised trials,[5,6,8,9] and several community-based studies.[10–12] A meta-analysis[13] concluded that topical repellents are ineffective in preventing malaria morbidity, with substantial differences in study design among the studies included in the final analysis (nine for *Plasmodium falciparum* and seven for *Plasmodium vivax*). The main limitation of these studies lies in the diversion of vectors from individual or household repellent users to non-users, spillover effects because of geographical contiguous communities, or follow-up periods immediately after the onset of the intervention.[14] A large-scale, cluster randomised trial attempting complete and continuous coverage of the vector control tool in the intervention group over two transmission seasons was expected to have a more uniform effect on malaria transmission. Moreover, wide-scale use of repellents in the community (cluster) can induce a reduction of the malaria burden by reducing human–vector contact,[15,16] the so-called, mass-effect.

In the Greater Mekong subregion, a diverse community of malaria vectors is present, several of which are known to feed outdoors and before nightfall, compromising the effectiveness of domicile vector control tools such as long-lasting insecticidal nets and indoor residual spraying.[17] We hypothesised that the synergy between indoor night-time protection of a long-lasting insecticidal net and outdoor evening protection of a topical repellent would further reduce the malaria transmission intensity, by affecting the vector population remaining outdoors. We aimed to assess the epidemiological efficacy of a highly effective topical repellent in addition to long-lasting insecticidal nets in the province of Ratanakiri, Cambodia, a region where vectors are known to show outdoor and early evening biting behaviour.[18]

## Methods

### Study design and participants

We did a cluster randomised controlled trial in the most endemic 117 of 240 villages in Ratanakiri province.[19] Average past incidence data (2010–11) were used to select the most endemic villages. Four villages were omitted

because of inaccessibility in the rainy season. Because of their proximity, another 25 villages were grouped into ten clusters. Hence, the study took place in 98 clusters, consisting of either a single village or a group of neighbouring villages. According to a 2012 population census, which was updated in 2013, the total population residing in the selected study villages was 48 838 individuals. The province is largely inhabited by Indigenous groups such as the Jarai, Kreung, and Tumpuon, as opposed to the Khmer in the rest of Cambodia. They generate revenue by subsistence slash-and-burn farming on plots located near or in the forest and less frequently on wet rice fields. During the rainy season most of them leave the village and stay on their plot farm.[20]

The study was organised as a cluster randomised trial with two groups (figure 1). All communities received a long-lasting insecticidal net (Olyset, Sumitomo Chemicals, Japan), with one net provided per person. Additionally, all households in the intervention group were provided with topical repellents (picaridin KBR3023, SC Johnson, Racine, WI, USA) during the malaria season (April–December) following a biweekly repellent distribution schedule.[21] Children (aged ≤10 years) were provided with a 10% milky lotion formulation, and adults a 20% spray formulation. The content of the active ingredient (picaridin 10% or 20%) and relevant impurities were in agreement with the WHO specifications. Entomological efficacy of the topical repellent was high against indigenous primary and secondary malaria vectors (>95% protection for at least 5 h).[22]

The study protocol (available on request) was approved by the Institutional Review Board of the Institute of Tropical Medicine Antwerp (approval IRB/AB/ac/154), the Ethics Committee of the University of Antwerp (approval B300201112714), and the Cambodian National Ethics Committee on Health Research (approval 265 NECHR). Village leaders provided written informed consent for the participation of their community in the trial. Individual written informed consent was obtained from all participants in the surveys. Parents provided consent for children younger than 10 years. All personal information was anonymised using reference numbers, and individual names were removed from the final database. Confidentiality agreements were signed by all participating research teams. A reproducible data analysis report in R markdown format including data and R code for the primary outcome will be made available in an online public repository. Secondary outcome measures are currently used by PhD students for further research and will be made available upon request.

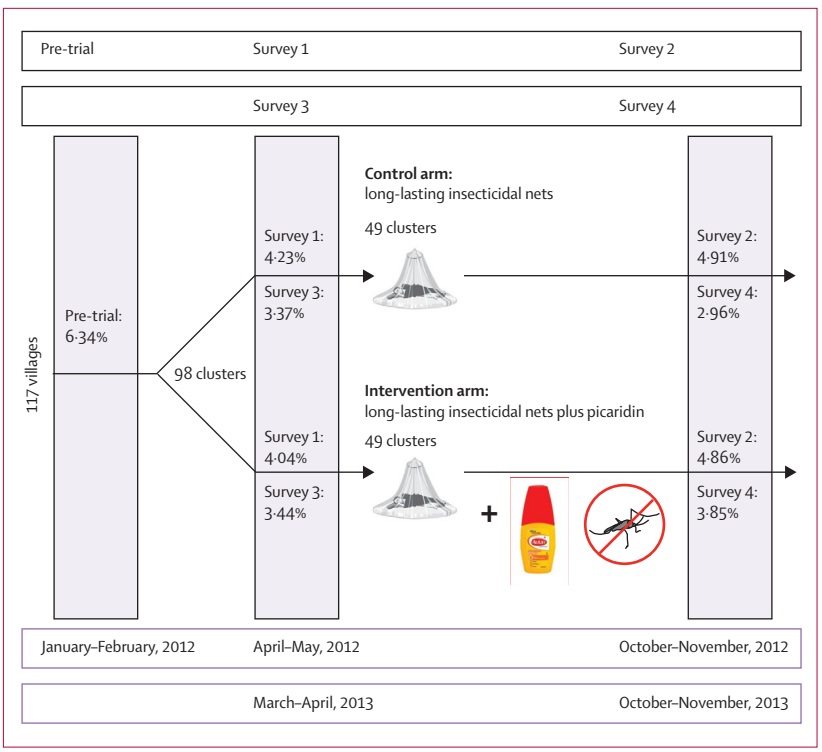


*Figure 1:* **Design of the cluster randomised trial with two study groups. Grey boxes indicate the timing of the surveys during which the primary endpoint (PCR prevalence) was obtained**

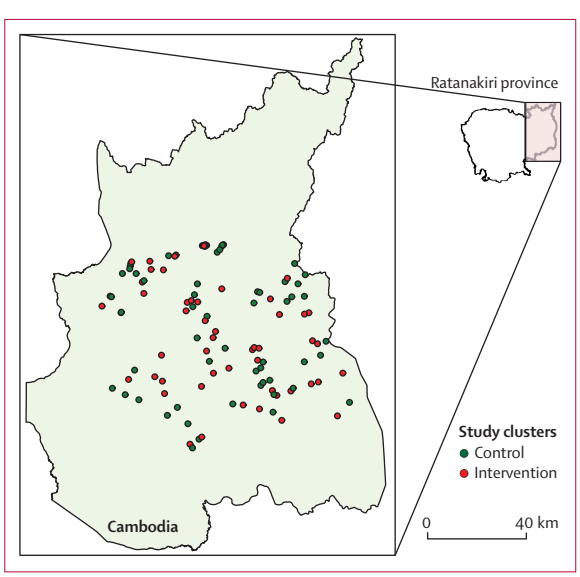


*Figure 2:* **Location of the communities allocated to treatment group by restricted randomisation in Ratanakiri province, Cambodia**

For the **online public repository** see https://zenodo.org/record/55711

## Randomisation and masking

The 98 clusters were randomly assigned (1:1) to either receive long-lasting insecticidal nets (control) or long-lasting insecticidal nets plus topical repellent (intervention). Randomisation was constrained taking into account eight different restriction factors to ensure a spatially well balanced study design (figure 2). Data from the pre-trial study[19] were used to restrict randomisation by prevalence, participant response rate, and equity of clusters having zero prevalence. Additionally, past incidence (2010–11), population size, presence of

village malaria workers, minimum distance to the nearest community (>1500 m), and approximate geographical balance were used as balance criteria.[23] A restriction factor for the combined set of criteria was estimated from 1 000 000 randomly generated allocations and expressed as the proportion of acceptable allocations. Validity of the design of the restricted allocation process was investigated by verifying whether each pair of communities had the same probability of being allocated to the same treatment according to the balance criteria.[23]

### Procedures

Five cross-sectional surveys were done over a 2 year period. The first survey, a pre-trial study, was done between Jan 31, 2012, and Feb 24, 2012, and used to obtain baseline information about malaria prevalence at the community level.[19] Four additional cross-sectional surveys were done at the beginning and end of the transmission season (survey 1 between April 24, 2012, and May 16, 2012; survey 2 between Oct 22, 2012, and Nov 13, 2012; survey 3 between March 19, 2013, and April 10, 2013; and survey 4 between Oct 18, 2013, and Nov 9, 2013; figure 1). Each survey consisted of a 2-day sampling period per cluster to reach 65 participants randomly selected from the 2012 population census, to have a representative sample of the community (cluster). In case of a low response rate from the initially selected participants, an additional list of 15 randomly selected individuals was provided to the survey teams until they reached a minimum of 50 participants (figure 3). This list of participants was provided 1 day in advance to the village chief, and many of the participants came to the village centre to collaborate. Others remained in their fields or village houses. A team was sent out to search for them, some were found, others could not be located within the 2 day sampling window.

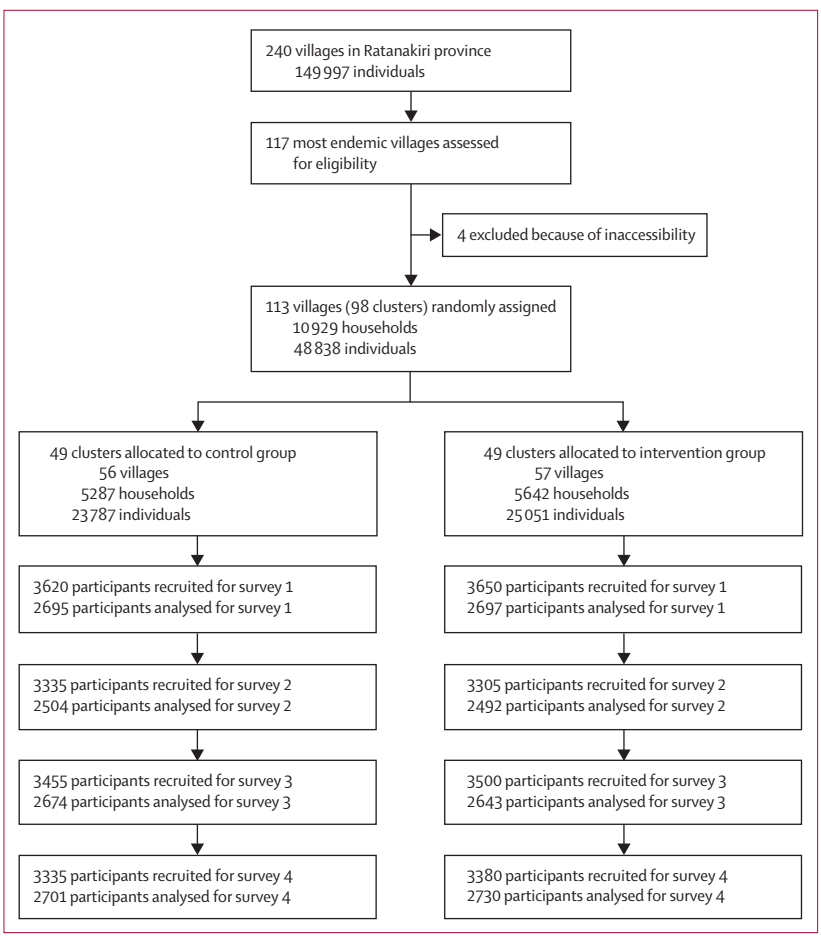


***Figure 3:*** **Trial profile**
The recruited participants not analysed were not present during the 2 day sampling period in each cluster.

A capillary tube was used to collect blood samples of participating individuals from a fingerprick. Blood was collected every day during each survey. 5 µL of blood was stored immediately in a labelled 96-well plate containing Whatman 3MM filter paper. Additionally, two back up blood spots of 20 µL were stored on similar filter paper. Baseline information, such as age, sex, and ethnicity of all the participants was collected in every survey alongside self-reported information about long-lasting insecticidal nets (survey 3 and 4) and repellent use (surveys 2, 3, and 4). Health promotion campaigns were organised during the entire study period in all communities and in both study groups. Use of long-lasting insecticidal nets was promoted in the control and intervention group, whereas repellent use was promoted in the intervention group only. In the first year, the health promotion campaign methods used were oral information, leaflets, and posters, whereas in the second study year, visual media were additionally used to stimulate the use of net and repellent control measures.[21]

The repellent consumption rate was measured per family every 2 weeks during the repellent distribution by visual inspection of the leftover repellent divided into categories (eg, empty, half full, full).[21] A proxy of daily compliance was estimated by self-reporting and observation. Participants were probed during surveys 2, 3, and 4 with a standard questionnaire (eg, "did you use repellent yesterday/last week?"). Self-reported compliance to long-lasting insecticidal nets was queried in survey 3 and 4 with the question, "Did you sleep under a long-lasting insecticidal net last night?" Between survey three and four, a social science study was done to assess the acceptability and use of repellents in ten selected clusters.[20]

### Outcomes

The primary endpoint was species-specific malaria prevalence obtained through real-time PCR analysis using the method described by Canier and colleagues.[24] Prevalence was calculated for participants infected with any of the malaria species from the *Plasmodium* genus (*Plasmodium* spp), or separately, for participants infected with either *P falciparum* or *P vivax*. *Plasmodium*

*malariae* and *Plasmodium ovale* were not reported separately because of low endemicity. Secondary outcomes were incidence and malaria exposure measured by serological markers. Incidence was measured through the passive case detection of the national health system (ie, health centre or hospital visits or village malaria workers)[25] and a battery of 21 malaria-specific antigens was used as biomarkers for malaria exposure. A multiplex serological assay was done in a subset of 2000 random blood samples from survey 2 and 4.[26] Antibody half-life was assessed with seroreversion rates[27] and mixed linear regression models for repeated measurements data (appendix p 2). This information was used as a reference to guide which malaria or vector antigens could provide insight into short-term immunological trends, hence allowing for comparison between treatment groups.[28] Individuals who tested positive for PCR were visited, informed of their status, and treated free of charge within 24–48 h according to the Cambodian National Malaria Treatment guidelines. Complete safety analysis data will be published seperately; any ad-hoc adverse events are reported here.

### Statistical analysis

The required sample size, taking into account a 30% individual dropout rate because of the foreseen difficulties in finding participants within a 2 day window, was estimated at 3100 participants per treatment group, divided over 50 communities. The calculation was done to detect a 40% reduction in an expected PCR prevalence of 5% from the control group with a power of 80% and a between-cluster coefficient of variation of k=0·5 using the formula described by Hayes and Moulton.[23] A retrospective power analysis was done with the same formula, but with a coefficient of variation estimated from the data.

Comparison of primary and secondary outcomes between treatment groups was done with statistical methods, allowing for individual-level adjustments for age and sex, but also taking into account the clustered nature of the data. The PCR prevalence between the study groups was compared with a generalised estimation equation with an exchangeable correlation structure to account for the cluster-based design. For the intention-to-treat analysis (including all participants present for the respective surveys), data were examined per survey and adjusted for age and sex as specified a priori in the statistical analysis plan. The per-protocol analysis (including only the participants reporting to have used their net the day before [control group] or have used their bednet and applied repellent [intervention group] the day before present for the survey) was done in a subset of participants from the final survey (survey 4). Unadjusted and adjusted analyses for age and sex were done on the subset of data. Incidence per 1000 person-months was calculated as the episodes per person per month at risk. Comparison of treatment groups for secondary outcomes was done with linear mixed models, taking into account the clustered nature of the study design and the appropriate error distribution; Poisson was selected for analysis of the incidence data and Gaussian was used to calculate the log-transformed median fluorescent intensity of the serological markers. Averages of duplicated serological measures were obtained after outliers had been removed, according to the algorithm described by Eo and colleagues.[29]

All information was entered in an Access database with preprogrammed forms to minimise data entry errors by two independent data entry clerks. Standard double data checks were made and mismatches were corrected based on hard copies. Each individual was anonymised via a ten digit code and the PCR results were digitally merged with the survey data based on this unique code. The study was monitored by a data safety and management board. All statistical analyses were done with software package R version 3.1.3. Literate programming techniques and markup language were used to ensure maximum reproducibility.[30] This trial is registered with ClinicalTrials.gov, number NCT01663831.

See Online for appendix

### Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

| | Control (n=2695) | Intervention (n=2697) |
|---|---|---|
| Age (years) | 18 (9–32) | 19 (8–33) |
| Female | 1408 (52%) | 1352 (50%) |
| Male | 1285 (48%) | 1345 (50%) |
| Risk behaviour | | |
| Plot hut | 990 (37%) | 1235 (46%) |
| Forest | 244 (9%) | 412 (15%) |
| Ethnic origin | | |
| Jarai | 769 (29%) | 679 (25%) |
| Tumpuon | 682 (25%) | 713 (27%) |
| Kreung | 408 (15%) | 672 (25%) |
| Prov | 230 (9%) | 188 (7%) |
| Other | 599 (22%) | 426 (16%) |
| Infection status | | |
| *Plasmodium spp* | 114 (4·23%) | 109 (4·04%) |
| *Plasmodium falciparum* | 54 (2·00%) | 44 (1·63%) |
| *Plasmodium vivax* | 69 (2·56%) | 85 (3·15%) |

Data are median (IQR) or n (%). The number of participants excluded missing information for the characteristic of interest. Blood samples were collected in 2695 participants in the control group and 2697 in the intervention group. Plot hut and forest refer to the overnight locations of participants.[19,20]

***Table 1:*** **Baseline characteristics of participants and PCR diagnostics per treatment group in survey 1**

| | Control | Intervention | OR (95% CI) | p value | aOR (95% CI) | p value |
|---|---|---|---|---|---|---|
| **Survey 1** | | | | | | |
| *Plasmodium* spp | 114/2695 (4·23%) | 109/2697 (4·04%) | 0·961 (0·574–1·608) | 0·878 | 1·052 (0·625–1·770) | 0·849 |
| *Plasmodium falciparum* | 54/2695 (2·00%) | 44/2697 (1·63%) | 0·807 (0·448–1·451) | 0·473 | 0·811 (0·445–1·478) | 0·493 |
| *Plasmodium vivax* | 69/2695 (2·56%) | 85/2697 (3·15%) | 1·240 (0·719–2·139) | 0·440 | 1·372 (0·803–2·345) | 0·248 |
| **Survey 2** | | | | | | |
| *Plasmodium* spp | 123/2504 (4·91%) | 121/2492 (4·86%) | 0·987 (0·591–1·646) | 0·959 | 1·008 (0·598–1·702) | 0·975 |
| *P falciparum* | 49/2504 (1·96%) | 63/2492 (2·53%) | 1·308 (0·722–2·370) | 0·376 | 1·316 (0·727–2·384) | 0·364 |
| *P vivax* | 86/2504 (3·43%) | 63/2492 (2·53%) | 0·724 (0·416–1·259) | 0·252 | 0·744 (0·415–1·333) | 0·320 |
| **Survey 3** | | | | | | |
| *Plasmodium* spp | 90/2674 (3·37%) | 91/2643 (3·44%) | 1·040 (0·613–1·764) | 0·884 | 1·087 (0·643–1·838) | 0·755 |
| *P falciparum* | 33/2674 (1·23%) | 36/2643 (1·36%) | 1·130 (0·545–2·341) | 0·743 | 1·179 (0·560–2·483) | 0·664 |
| *P vivax* | 71/2674 (2·66%) | 58/2643 (2·19%) | 0·822 (0·488–1·386) | 0·462 | 0·842 (0·497–1·426) | 0·523 |
| **Survey 4** | | | | | | |
| *Plasmodium* spp | 80/2701 (2·96%) | 105/2730 (3·85%) | 1·296 (0·805–2·088) | 0·286 | 1·311 (0·814–2·111) | 0·266 |
| *P falciparum* | 36/2701 (1·33%) | 30/2730 (1·10%) | 0·814 (0·433–1·529) | 0·523 | 0·829 (0·440–1·561) | 0·561 |
| *P vivax* | 50/2701 (1·85%) | 73/2730 (2·67%) | 1·448 (0·846–2·478) | 0·177 | 1·505 (0·883–2·566) | 0·133 |

Data are n/N (%), unless otherwise specified. No significant differences between control and intervention group were noted. OR=odds ratio. aOR=adjusted odds ratio.

***Table 2*: Real-time PCR prevalence results of all surveys in 2012 and 2013 for the intention-to-treat analysis**

| | Species | Control | Intervention | IRR (95% CI) | p value |
|---|---|---|---|---|---|
| 2012 | *Plasmodium* spp | 99·76 (2331/23 366) | 87·55 (2176/24 854) | 0·920 (0·652–1·298) | 0·636 |
| 2012 | *Plasmodium falciparum* | 43·57 (1018/23 366) | 38·99 (969/24 854) | 0·887 (0·885–0·890) | 0·730 |
| 2012 | *Plasmodium vivax* | 31·88 (745/23 366) | 28·20 (701/24 854) | 0·885 (0·566–1·384) | 0·594 |
| 2013 | *Plasmodium* spp | 51·16 (1217/23 787) | 45·79 (1147/25 051) | 0·943 (0·642–1·385) | 0·766 |
| 2013 | *P falciparum* | 21·82 (519/23 787) | 17·96 (450/25 051) | 0·656 (0·316–1·360) | 0·256 |
| 2013 | *P vivax* | 15·26 (363/23 787) | 16·69 (418/25 051) | 0·982 (0·627–1·535) | 0·934 |

Data are incidence (episodes of malaria/total person-months at risk), unless otherwise specified. IRR=incidence rate ratio.

***Table 3*: Incidence (per 1000 person-years) of symptomatic malaria cases as reported by the health system in the control group versus the intervention group**

## Results

During the malaria season of 2012, 71726 bottles of 100 mL repellent were distributed, averaging 2·9 bottles per person per year. Intensification of promotional and organisational efforts in the second study year (2013) resulted in increased distributor–family contact rates, leading to the distribution of 223 510 bottles in 2013 and averaging 8·9 bottles per person per year.[20] After the restricted randomisation, both baseline prevalence and characteristics were found to be well balanced between the study groups (tables 1 and 2; figures 1 and 2). The combined restriction factor was estimated to be 99·13% (95% CI 99·11–99·15), whereby a total of $2·215 \times 10^{26}$ acceptable allocations remained.

PCR prevalence of malaria in the respective surveys was 4·23% (survey 1), 4·91% (survey 2), 3·37% (survey 3), and 2·96% (survey 4) in the control group and 4·04% (survey 1), 4·86% (survey 2), 3·44% (survey 3), and 3·85% (survey 4) in the intervention group. Self-reported use of long-lasting insecticidal nets was over 90% and equal among study groups, taking into account the cluster effect (survey 3: $\chi^2$=0·84, p=0·36; survey 4: $\chi^2$=1·81, p=0·18). After repellent distribution rounds in survey 2 and survey 4, 72% of participants in the intervention group and 69% of participants in the control group reported using the repellent the day before they participated. In the control group, where no repellent distribution took place, these figures drop to 0·36% for survey 2 and 0·19% for survey 4.

The primary outcome measure of malaria prevalence did not differ between control and intervention groups in any of the follow-up surveys taking into account the cluster effect (table 2). Repeating the analysis adjusted for age and sex did not alter the results. Secondary outcome measures confirmed the statistical results of the primary outcome. Annual incidence rates for all *Plasmodium* species combined, or separately for confirmed *P falciparum* or *P vivax* cases did not differ between treatment groups, neither in 2012 nor in 2013 (table 3). The median fluorescent intensity of the serological markers for *P falciparum*, *P vivax*, and *P malariae* did not differ between treatment groups (appendix p 1).

4416 (81%) of 5431 individuals were selected for the per-protocol analysis. In the control group, 2570 (95%) of 2701 participants reported to sleep under their bednets. In the intervention group, 1846 (68%) of 2730 participants reported to both apply their topical

repellents and sleep under their bednets the day before the last survey. No additional protective effect was shown comparing the prevalence endpoints between the control and intervention group using the individual-level unadjusted and age-adjusted and sex-adjusted analysis taking into account the cluster effect (table 4).

833 participants were recorded as being infected with plasmodium of any species during the four surveys. Of these, 814 (98%) took treatment in front of the village malaria worker or provincial health department field staff. Standard 3-day treatment with dihydroartemisinin-piperaquine was provided to 748 (92%) infected participants, and 66 (8%) received artesunate-mefloquine. Six pregnant women were referred to a health centre for further treatment.

Over the 2-year study period, 41 ad-hoc adverse event notifications from nine villages were received. After the visit by the project medical doctor, 33 events were classified as adverse reactions, of which 11 were cases of repellent abuse through oral ingestion, either accidental or not. All participants with adverse reactions fully recovered and 17 of them were advised to permanently stop using the repellent. Additionally, a monitoring system at the family level was put in place to document perceived side-effects.

| | Control (n=2570) | Intervention (n=1846) | OR (95% CI) | p value | aOR (95% CI) | p value |
|---|---|---|---|---|---|---|
| *Plasmodium spp* | 2·68% | 4·01% | 1·454 (0·847–2·497) | 0·175 | 1·499 (0·870–2·583) | 0·145 |
| *Plasmodium falciparum* | 1·17% | 0·98% | 0·815 (0·407–1·633) | 0·564 | 0·820 (0·407–1·651) | 0·579 |
| *Plasmodium vivax* | 1·63% | 2·87% | 1·712 (0·922–3·181) | 0·089 | 1·790 (0·966–3·318) | 0·064 |

Unadjusted OR and aOR statistics with 95% CI were reported. OR=odds ratio. aOR=adjusted odds ratio.

*Table 4:* **Results of the per-protocol analysis of the real-time PCR prevalence for survey four**

## Discussion

This cluster randomised trial, designed to detect population-level rather than individual effects, did not show a reduction in malaria infection and disease in the intervention group. When compared with earlier cluster-based intervention studies of topical repellents, this trial was scaled up by a factor of six, incorporating 49 clusters (communities) in each treatment group. All existing homesteads had continuous access to repellents and received long-lasting insecticidal nets. Given the large number of randomised clusters, any confounder that might affect the measured outcomes was likely to be balanced between the two study groups, including the proportion of *P vivax* relapses, which are not affected by vector control tools.

The study, which was done in a multidisciplinary setting, aimed to cover the epidemiological, entomological, and anthropological aspects of the trial, obtained three epidemiological endpoints: PCR prevalence, incidence, and malaria exposure measured by serological markers. Similar trends were observed in these three outcomes and no indication for a supplementary protective effect provided by mass use of topical repellents in addition to long-lasting insecticidal nets on malaria prevalence was found. These results are in line with a recent meta-analysis[13] of household and small community randomised trials in which repellents were found unlikely to provide effective protection against malaria. As previously reported,[22] the picaridin repellent product showed high individual protective efficacy against bites from malaria vectors in the same area. However, in the 4788 anopheles mosquitoes that were collected in two control and two intervention villages during 2240 human landing collection nights by untreated collectors, no *P falciparum* and *P vivax* sporozoite carriers were detected by circumsporozoite protein enzyme-linked immunosorbent assay (Durnez L, Institute of Tropical Medicine, Belgium, unpublished). Additionally, a social science work package investigated the sociocultural factors affecting the effectiveness of the intervention, including accessibility, acceptance, and actual daily skin application of the repellent.[20,21]

Three limiting factors of the present study design might have hindered the rejection of the null hypothesis of there being no difference between treatment groups. First, the study was powered to detect a 40% difference in an expected PCR prevalence of 5% from the control group with a cluster coefficient of variation k=0·5 and a power of 80%. A retrospective power analysis, based on the observed data (ie, higher estimated k of 1 and lower final prevalence of 3% in the control group), suggested that with a given power of 80%, the actual study design could only detect differences larger than 46% between study groups. Second, each individual that was found positive for malaria was treated according to the national guidelines within 24–48 h on detection by PCR, because it was considered unethical not to provide immediate treatment to the participants who tested positive for malaria. Given that this treatment was provided to participants randomly selected for the cross-sectional surveys only and equally in both study groups, it was expected to have a negligible effect on the study outcome. Lastly, although self-reported repellent consumption was high (72% in survey 2 and 69% in survey 4 in the intervention group), and contamination of the control clusters was low (0·36% in survey 2 and 0·19% in survey 4 in the control group), repellent use might not have been optimum. An independent anthropological study parallel to the trial, using both quantitative and qualitative methods,[20] found that 59% of the respondents of a cross-sectional survey done in all intervention clusters reported to use the repellent 7 days a week. Observational studies done in a small selection of ten clusters in the intervention group resulted in

evening skin application rates between 6% and 15%.[20] Considering the difficulty in obtaining an unbiased estimate of actual repellent skin application at the community level, our best estimate lies between what was self-reported (70%) and what was observed in a small number of clusters (15%). According to the qualitative component of the same observational study, repellent use was highly variable and alternative uses other than skin applications were common. Although these results indicate that daily repellent use was suboptimum, it was consistently higher in men during forest-related activities. This was partly because of perceived insect nuisance while performing activities such as slash-and-burn farming or hunting in the forest. Although repellent use improved over the course of the intervention as a result of intensive and continuous promotion of appropriate use of the repellent in the intervention clusters, it remained suboptimum and highly variable.

This cluster randomised trial, designed to assess the epidemiological efficacy of community-wide use of topical repellents in addition to long-lasting insecticidal nets, did not show a reduction in malaria infection and disease in the intervention group. Although individual protective efficacy of repellents against malaria vectors remains guaranteed, the results of this cluster-based trial support the conclusion that community-wide distribution of highly effective topical repellents might not contribute to a further decrease of malaria endemicity in a pre-elimination setting. Daily compliance and appropriate use of the repellents, achieved under optimum trial conditions with sufficient resources to promote and distribute the repellent product, remain the main obstacles. We conclude that mass distribution of topical repellents in addition to long-lasting insecticidal nets has no added public health value in preventing malaria in low endemic countries from the Greater Mekong sub-region.

#### Contributors

LD, VS, DM, and MC designed the study. VS, SH, LD, SK, KVR, CG, SM, SU, SS, TS, DM, and MC organised the field work. SH, SK, VS, LD, CG, and KVR collected the data. LD, LC, SK, NK, KK, and DM organised and did the lab work. VS did the statistical analysis and prepared for online data sharing. VS, LD, CG, SH, KPG, DM, and MC wrote the paper. MC is guarantor.

#### Declaration of interests

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