Tropical Medicine and International Health doi:10.1111/j.1365-3156.2006.01673.x

VOLUME 11 NO 8 PP 1166–1177 AUGUST 2006

Insecticide-treated bednets for the prevention of *Plasmodium falciparum* malaria in Cambodia: a cluster-randomized trial

T. Sochantha[1], S. Hewitt[2], C. Nguon[1], L. Okell[3], N. Alexander[3], S. Yeung[3], H. Vannara[4], M. Rowland[3] and D. Socheat[1]

1 *National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia*
2 *VBDC Consulting Ltd, Llandysul, UK*
3 *London School of Hygiene and Tropical Medicine, London, UK*
4 *Provincial Health Department, Rattanakiri, Cambodia*

Summary

OBJECTIVES To validate and quantify the impact of insecticide-treated bednets (ITN) on malaria morbidity and mortality in Cambodia.

METHODS A paired, cluster-randomized trial of ITN was conducted in Rattanakiri, North East Cambodia. Thirty-four villages with a total population of 10 726 were randomized to receive deltamethrin-impregnated bednets or to control (no net provision). Cross-sectional surveys measured *Plasmodium falciparum* prevalence at baseline and 10 months after ITN distribution. Village malaria volunteers in control and intervention villages treated dipstick-positive *P. falciparum* cases with artesunate and mefloquine. The resulting passive surveillance data were used as an estimate of the incidence of clinical *P. falciparum* infections.

RESULTS There was a protective efficacy of 28% in *P. falciparum* incidence (adjusted rate ratio 0.72, 95% CI 0.47–1.08) and 9% in *P. falciparum* prevalence (adjusted prevalence ratio 0.91, 95% CI 0.65–1.28) in ITN relative to control villages; however, neither of these estimates reached statistical significance. Individual-level analysis indicated a greater reduction in *P. falciparum* prevalence among under 5-year-olds (adjusted OR = 0.63, 95% CI 0.26–1.53) compared to older individuals (interaction $P = 0.042$). The protective efficacy of 35% (95% CI –28, 67%) with respect to clinical *P. falciparum* incidence in under 5-year-olds was more pronounced than the corresponding estimates for prevalence but was again not significant.

CONCLUSIONS Lack of statistical significance in the results is likely to be due to a lack of power. The analysis provides further evidence for ITN effectiveness in South East Asia, particularly among individuals under 5 years of age.

keywords insecticide, bednet, malaria, *Plasmodium falciparum*, Cambodia, trial

Introduction

Insecticide-treated bednets (ITN) are widely used as protection against malaria in endemic regions and are a major component of the World Health Organization Roll Back Malaria strategy for reducing malaria-related morbidity and mortality. A meta-analysis of randomized controlled trials concluded that ITN interventions are highly effective against malaria in most tropical and subtropical regions (Lengeler 2004). In areas of stable malaria where the annual entomological inoculation rate (EIR) (the number of infectious bites per person per year), is >1, ITN were associated with a protective efficacy (i.e. relative reduction) in the incidence of uncomplicated *Plasmodium falciparum* clinical episodes of approximately 50% and a proportional reduction of *P. falciparum* prevalence of 13%. Particular benefit was observed in children under 5 years of age, with a relative reduction in all-cause mortality of 17%. But most of these trials were carried out in Africa, Latin America and South Asia. There are fewer studies and no large-scale randomized trials from South East Asia, where differences in vector biting cycles and malaria epidemiology raise questions about whether a similar impact would be achievable.

The Cochrane review of ITN studies identified two randomized controlled trials conducted in South East Asia (Lengeler 2004). On the Thailand–Myanmar border, children aged 4–15 who were given ITN had 41% fewer

symptomatic episodes and a non-statistically significant 20% relative reduction in the prevalence of *P. falciparum* when compared with those with untreated bednets (Luxemburger *et al.* 1994). A study in eastern Thailand showed a 41% reduction in the incidence of mild clinical episodes of *P. falciparum* and *Plasmodium vivax* in migrant workers supplied with ITN when compared with untreated bednets (Kamol-Ratanakul & Prasittisuk 1992). These were in areas of unstable malaria (EIR < 1).

There is a need to further validate these findings with larger randomized controlled trials, and expand evidence to include other South East Asian settings, since malaria epidemiology, transmission intensity and vector species, all of which affect ITN impact, show considerable local variation (Trung *et al.* 2004). Furthermore, the Cochrane review of ITN trials did not identify any previous cluster-randomized trials in South East Asia (Lengeler 2004). These are needed to show whether the vector population is susceptible to the 'mass-killing' effect, which is believed to account for the high impact of ITN in some of the previous cluster-randomized trials in Africa (Binka *et al.* 1998; Howard *et al.* 2000; Hawley *et al.* 2003). There is some evidence that ITN have a larger relative protective effect in areas with lower levels of transmission, and therefore from this point of view may be expected to be more effective in South East Asia than in Africa where transmission intensities are very high in some areas (EIRs of 100–500) (Lengeler 2004).

In Cambodia, malaria transmission occurs mainly in remote, sparsely populated, forested areas, as in many South East Asian countries (National Center for Parasitology Entomology and Malaria Control 2001). Here, transmission is perennial, with a peak during the rainy season (Brown *et al.* 2002; Bury unpublished data). ITN are a major focus of the national malaria control strategy (National Center for Parasitology Entomology and Malaria Control 2001) and present a potentially suitable intervention for high-risk populations who often live in impoverished and inaccessible regions with poor access to effective treatment. The main malaria vector in forested regions of Cambodia is *Anopheles dirus*, with *Anopheles minimus* playing a lesser role (Meek 1995). Both vectors are highly susceptible to the pyrethroid insecticides commonly used on ITN, such as deltamethrin (Curtis 1991; Blonsky & Rowland 2002). Compared with the most important African vector, *Anopheles gambiae*, *A. dirus* begins to bite earlier in the evening, from around 1900 hours (depending on the subspecies), and is more exophagic and exophilic (more often bites and rests outdoors) (Baimai *et al.* 1988; Blonsky & Rowland 2002; Warrell 2002; Trung *et al.* 2004). Both these factors may limit the impact of ITN in this setting, particularly the potential for mass-killing of the vector population (Meek 1995). Here, we report the results of a cluster-randomized trial conducted between 2001 and 2002 in rural villages in Cambodia to evaluate the effect of deltamethrin-impregnated bednets on *P. falciparum* malaria incidence and prevalence.

Methods

Study area and population

The trial location was Rattanakiri province, North East Cambodia, a remote, forested, mountainous area bordering Vietnam and Laos. The 36 villages initially selected for inclusion in the study (Figure 1) had not been supplied with ITN under the national malaria control programme. Most of the people are semi-nomadic subsistence farmers who frequently spend nights away from the villages to tend to their rice fields from January to August or to obtain materials from the forest in any season. Poverty levels are high, with the under-5 mortality rate at 229 per 1000 (National Institute of Statistics & Directorate General for Health 2001). Malaria is a major cause of morbidity and mortality in the area, accounting for an estimated 9.5% of all inpatient consultations (Bury unpublished data). A small-scale study in the north eastern province of Rattanakiri found an annual EIR of 6.0, of which *A. dirus* was estimated to contribute 5.2. Only subspecies A has been implicated, this species biting from around 1900 hours to a peak at 2100–2400 hours (Baimai *et al.* 1988; Blonsky & Rowland 2002; Trung *et al.* 2004). In 2001, 72% of confirmed malaria cases were *P. falciparum*, 26% *P. vivax* and 2% a mixture of the two (National Institute of Statistics & Directorate General for Health 2001). Geographical isolation and lack of a common language can make it difficult to access the public health sector. Private drug sellers are the main source of first-line treatments to an estimated 90% of malaria patients, which often contain little or no active ingredient against malaria (Bury unpublished data).

Study design

Prevalence of *P. falciparum* infection was selected as the primary outcome. Study villages were paired according to baseline prevalence, then randomized to receive ITN or to control using computerized random number generation. The sample size was originally calculated aiming for 90% power to detect a 50% relative reduction in prevalence from 30% to 15%. A standard deviation of 20% in absolute prevalence was assumed.

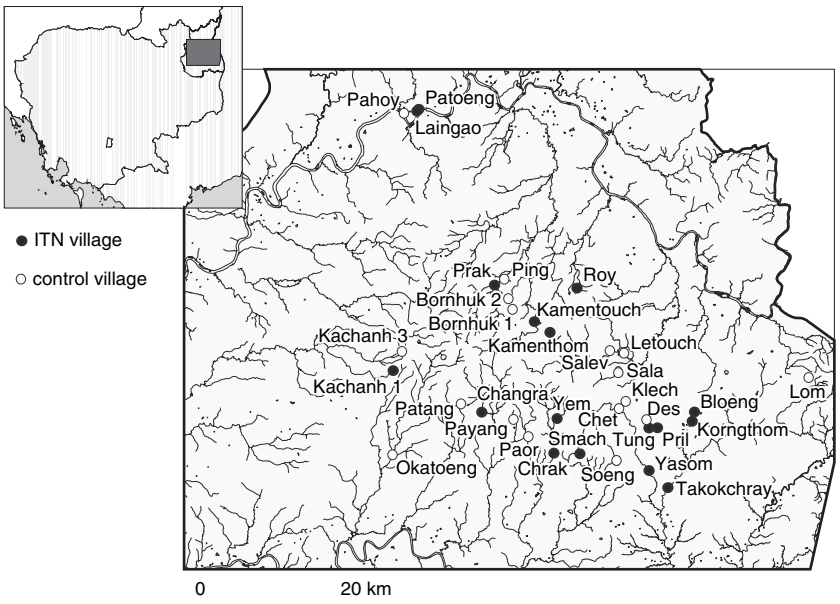

Figure 1 The study area and villages in Rattanakiri province and their location in Cambodia (top left); adapted with kind permission from Cox *et al.* 2004.

Treatment intervention and passive surveillance

To minimize risk to the communities involved, treatment services in the form of volunteer village malaria workers were set up in both control and intervention villages in June 2001. This allowed a 5-month establishment phase prior to ITN distribution. One volunteer was recruited per village and trained to recognize malaria symptoms, conduct rapid diagnostic tests (RDT) using Paracheck *P. falciparum*® (Orchid Biomedical Systems, Goa, India) and administer treatment. The sensitivity and specificity of the Paracheck *P. falciparum* are high at 92–97% and 88–97%, respectively (Proux *et al.* 2001; Guthmann *et al.* 2002). In another study, village malaria workers using RDT in rural areas of Laos interpreted more than 98% of results correctly (Mayxay *et al.* 2004). Villagers could consult the village malaria worker when unwell and if tested positive, they were treated with antimalarials. Table 1 gives details of the dosing schedule. Village malaria workers initially prescribed artesunate or mefloquine monotherapy, but in September 2001 they switched on to using artesunate and mefloquine combination therapy according to the simplified national guidelines when pre-packaged drugs became available. Where necessary, patients were referred to the district health centre. The village malaria workers collectively acted as a passive surveillance system, recording the age and sex of the patient, the test result and the treatment given. Records were cross-checked against medicine stocks and cassettes of used RDTs. This provided a proxy measure of *P. falciparum* incidence. At the end of the trial, the village malaria worker programme was maintained in the study villages and expanded to cover other remote communities.

Table 1 Treatment protocols adapted from the Cambodian national treatment guidelines for uncomplicated *Plasmodium falciparum* malaria and used by village malaria workers

			Doses of artesunate (A) (50 mg) and mefloquine (M) (250 mg)				
			Day				
Time period	Age (years)	Delivery	1	2	3	4	5
June–August 2001	<6	Rectocap	1A	1A	1A	1A	1A
	6–10	Tablet*	2M	–	–	–	–
	11–14	Tablet*	3M	–	–	–	–
	>14	Tablet*	4M	–	–	–	–
September 2001 onwards	<6	Rectocap	1A + 1/2M	1A	1A	1A	1A
	6–10	Tablet*	2A + 2M	2A	2A	–	–
	11–14	Tablet*	3A + 3M	3A	3A	–	–
	>14	Tablet*	4A + 4M	4A	4A	–	–

* If patient unable to swallow, give artesunate suppository and refer to health centre.

Cross-sectional surveys

Malaria prevalence was measured by cross-sectional surveys of all study villages in September 2001, prior to ITN distribution, and again at the end of the study in September 2002. Village population estimates were obtained from up-to-date census lists owned by village heads. Teams aimed

to sample 250 people per village by random selection of numbered households, from which all present were sampled. If the total population of the village was under 250, the target sample was set at 80%. For each study participant, their age, sex and reported use of ITN the previous night were recorded and a blood sample obtained. Thick and thin blood smears were made for microscopy and individuals were classed as *P. falciparum*-positive if the slide showed asexual forms of the parasite. Individuals were not given identifiers or followed up between surveys.

ITN intervention

Polyester bednets (Siamdutch Mosquito Netting Co. Ltd, Bangkok, Thailand) were treated with deltamethrin at a concentration of 25 mg/m^2 and distributed to intervention villages in October 2001 in sufficient quantities to achieve 100% coverage of the population. New ITN were given to those already owning nets. At the end of the study, ITN were distributed to control villages and those in the ITN villages were reimpregnated.

Sociological and entomological studies

Health beliefs and knowledge related to malaria, treatment-seeking behaviour, use of ITN and vector insecticide resistance were surveyed in some of the study villages. The results were used to inform the current analysis and interpretation, although they are reported elsewhere (Blonsky & Rowland 2002; Brown *et al.* 2002).

Statistical analysis

Data were double-entered and study participants with missing slide results were removed from the data set. Slides were missing because of random loss or breakage during transit.

Plasmodium falciparum infection prevalence in each of the trial arms is reported as a geometric mean due to the right skew of the village-level prevalence values. The ratio of slide-positive *P. falciparum* prevalence in intervention when compared with matched control villages at the final cross-sectional survey was assessed initially at the cluster level with paired *t*-tests first on the unadjusted log prevalence values and then on values adjusted by logistic regression for age, sex, baseline prevalence and village population size (Bennett *et al.* 2002). The age groups used were 0–1, 2–5 years, 5-year bands up to age 40, 10-year bands up to age 70, and 71+ years. Baseline prevalence was included despite being the matching factor, because the matching was not precise (Table 3).

The effect of ITN on *P. falciparum* prevalence was also analysed by logistic regression on individual-level data, using a generalized estimating equation (GEE) approach (Liang & Zeger 1986). This allowed us to perform a test for interaction between age and the effect of ITN to further investigate the impact among children under 5 years of age. We used an exchangeable correlation matrix to adjust for clustering and estimated standard errors according to the method of Mancl and DeRouen (2001), which corrects for any bias caused by the relatively small number of clusters.

Plasmodium falciparum incidence was estimated for each village using passive surveillance data as a proxy measure to give a crude rate (i.e. the number of *P. falciparum*-positive consultations with village malaria worker per person per year). Village population estimates, which were obtained only at baseline, were used as denominators. Each person was assumed to become at risk at the start of the study and remain in the population at risk throughout because of the potential for multiple episodes per individual.

Plasmodium falciparum-positive consultation rates in intervention and control villages were compared using unadjusted and adjusted paired *t*-tests on the log rates. An adjusted rate ratio was obtained in two stages, first controlling for individual-level and then cluster-level potential confounders. Village *P. falciparum*-positive consultation rates were age–sex standardized by indirect methods, using rates in each age–sex stratum of the total study population as standards. Denominators were calculated from village population estimates assuming the age–sex distribution of the final cross-sectional survey, as this had fewer missing values than the baseline survey.

Scatter plots and linear regression were used to assess the nature of any association between village-level adjusted log *P. falciparum*-positive consultation rates and the cluster-level variables: village population size and baseline prevalence. Log linear models were used to control for these factors using multiple regression with log rates as the outcome, and ITN, pair and the potential confounders as the exposure terms in the model. The regression coefficient for the ITN term in the model is the adjusted log rate ratio.

Ethical approval

Ethical approval for this study was granted by Cambodia's Ministry of Health.

Results

Recruitment and randomization

Of the 36 villages initially included in the trial, one pair was excluded after the control village expressed a strong

Table 2 Baseline (2001) and post-intervention (2002) characteristics of the study villages by trial arm

	2001		2002	
Variable	ITN	Control	ITN	Control
Number of villages	17	17	17	17
Total population*	6106	4620	–	–
Mean village population*	359	272	–	–
Total sample size	2918	2905	2748	2646
Mean sample size per village	172	171	162	156
Age, median	15	15	16	13
Sex (% male)	46	47	47	47
Plasmodium falciparum prevalence† (%)	28	30	16	18
Reported ITN use in the previous night (%)	12	12	87	14

* Village populations were recorded only at baseline.
† Geometric mean over villages.

wish for ITN, which was granted. The remaining 34 villages had a total population of 10 726. The randomization procedure produced comparable baseline populations in the two trial arms in terms of average age, sex, prevalence of *P. falciparum* infection and reported ITN use by individuals (Table 2). By chance, mean village population size was larger in the ITN arm of the trial with an average of 87 more people per village, which is adjusted for during the analysis. Owing to the availability of only crude data at the time, the matching of villages on baseline infection prevalence is not precise. Nevertheless, the values were fairly similar within pairs (Table 3). After consulting a map of the area (Figure 1), the random allocations in three pairs were reversed to avoid spatial clustering.

Uptake of insecticide-treated bednets intervention

Reported ITN use suggested a high coverage in intervention villages, with the proportion sleeping under a net the previous night increasing from 12% at baseline to 87% at the second cross-sectional survey. By contrast, reported coverage in control villages went up from 12% at baseline to only 14%, indicating little contamination between trial arms. There was no significant difference in reported ITN use in under 5-year-olds when compared with the rest of the population in either the control or intervention villages (chi-square; $P = 0.873$ and 0.736, respectively) and no substantial variation in reported use in other age groups.

Table 3 Baseline and post-intervention *Plasmodium falciparum* prevalence, and unadjusted and adjusted prevalence ratios in matched pairs of ITN and control villages

	Baseline *P. falciparum* prevalence (2001)		Post-intervention *P. falciparum* prevalence (2002)			
Pair	ITN	control	ITN	control	Prevalence ratio unadjusted	Prevalence ratio adjusted for age, sex, village population size, baseline prevalence
1	0.10	0.28	0.05	0.12	0.38	0.82
2	0.14	0.18	0.08	0.19	0.43	0.48
3	0.18	0.20	0.09	0.05	1.81	2.27
4	0.16	0.17	0.09	0.04	2.26	1.92
5	0.25	0.23	0.09	0.28	0.32	0.32
6	0.27	0.20	0.12	0.23	0.50	0.36
7	0.31	0.28	0.24	0.15	1.61	1.21
8	0.31	0.26	0.49	0.19	2.59	2.03
9	0.30	0.35	0.15	0.22	0.68	0.81
10	0.33	0.33	0.20	0.29	0.67	0.65
11	0.37	0.36	0.14	0.46	0.30	0.28
12	0.39	0.32	0.17	0.17	0.99	0.81
13	0.40	0.36	0.31	0.15	2.09	1.70
14	0.51	0.43	0.28	0.19	1.43	1.12
15	0.46	0.57	0.22	0.33	0.68	0.88
16	0.39	0.37	0.23	0.22	1.03	1.17
17	0.39	0.50	0.25	0.18	1.38	1.85
Overall*	0.28	0.30	0.16	0.18	0.90 (95% CI 0.63–1.30) $P = 0.560$†	0.91 (95% CI 0.65–1.28) $P = 0.581$†

* Geometric means.
† *t*-test.

ITN effect on *Plasmodium falciparum* prevalence in the total study population

Between the September 2001 and September 2002 cross-sectional surveys, there was a decrease in the overall prevalence of slide-confirmed *P. falciparum* infection in both ITN and control arms from 29% to 17%. Age-specific prevalence is shown in Figure 2 by survey and trial arm. The crude prevalence ratio comparing ITN with control villages at the end of the trial was 0.90 (95% CI 0.63–1.30, $P = 0.560$) (Table 3). Adjusting for age, sex, village population size and baseline prevalence caused a negligible change in the overall prevalence ratio to 0.91 (95% CI 0.65–1.28, $P = 0.581$) (Table 3). This best estimate corresponds to a 9% protective efficacy, suggesting a small but non-significant effect of ITN. A similar level of significance was obtained from logistic regression GEE models using individual-level data. The odds ratio comparing prevalence in ITN to control villages was 0.81 (95% CI 0.38–1.74, $P = 0.586$) after adjusting for age group, sex, baseline prevalence and village population size.

ITN effect on *Plasmodium falciparum* incidence in the total study population

Analysis of passive surveillance data provided a proxy measure of clinical *P. falciparum* incidence during the ITN trial period (1 November 2001–31 August 2002). There were a total of 5127 village malaria worker consultations in the 34 study villages, 48.9% of which tested *P. falciparum*-positive. Figure 3 shows the rates of positive consultations by trial arm before, during and after the ITN trial period, and these suggest an increased utilisation of the village malaria workers over time and seasonality in incidence. Figure 4 shows the age-specific positive consultation rate by trial arm.

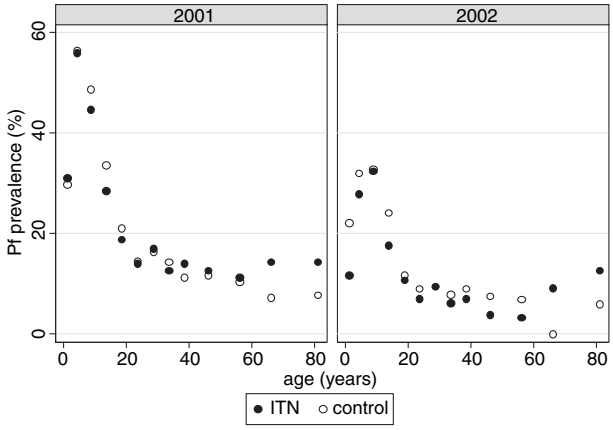

Figure 2 Age-group-specific prevalence at baseline (2001) and final (2002) cross-sectional surveys in the two trial arms. Values are plotted at the midpoint of the age group.

Baseline rates of *P. falciparum*-positive consultations calculated using data from the 5 months preceding the start of the trial were on an average 0.20 per person per year in ITN villages and 0.17 per person per year in control villages (geometric means of village rates). These were not significantly different ($P = 0.653$). During the 10-month trial period, the *P. falciparum*-positive consultation rate in ITN villages (0.21) was lower than that in control villages (0.36). This difference was borderline statistically significant, corresponding to an unadjusted rate ratio of 0.57 (95% CI 0.32–1.00, $P = 0.050$).

Standardizing for age and sex had little effect on the comparison between ITN and control villages (Table 4). Baseline *P. falciparum* prevalence was a strong predictor of the *P. falciparum*-positive consultation rate ($P < 0.001$), although adjusting for this factor had negligible effect on the estimate of effect of ITN as a result of the matching strategy. Village population size had a strong negative, linear association with the log age–sex-adjusted *P. falciparum*-positive consultation rates ($P < 0.001$). Adjusting for this factor reduced the magnitude of estimated ITN effect from the unadjusted rate ratio of 0.57 (95% CI 0.32–1.00) to 0.72 (95% CI 0.48–1.08) owing to the imbalance in village population size between trial arms.

The rate ratio after adjusting for all factors was 0.72 (95% CI 0.47–1.08, $P = 0.106$) (Table 4), corresponding to a protective efficacy of 28%, although this is again not statistically significant.

ITN effect on *Plasmodium falciparum* prevalence and incidence in children aged under 5 years

The overall difference in unadjusted *P. falciparum* prevalence in ITN relative to control villages was 6% in children under 5 years of age ($n = 999$) and 1.5% in those aged over 5 years ($n = 4392$). Logistic regression GEE models showed a statistically significant difference in adjusted odds ratios between ITN and control arms among under 5-year-olds (0.63, 95% CI 0.26–1.53) when compared with those over 5 years (0.90, 95% CI 0.43–1.88) (Wald test for interaction $P = 0.042$).

Analysis of *P. falciparum*-positive consultation rates in a data set restricted to children aged under 5 years showed that the adjusted rate ratio comparing ITN with control arms was 0.65 (95% CI 0.33–1.28, $P = 0.197$). This corresponds to a protective efficacy of 35%. Compared with the adjusted rate ratio of 0.86 (95% CI 0.66–1.13,

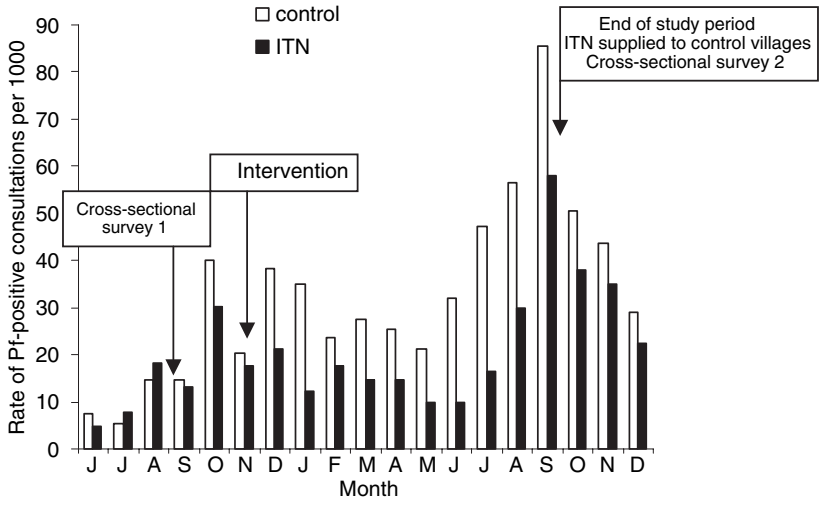

Figure 3 Rate of *Plasmodium falciparum*-positive consultations per 1000 per month comparing control to ITN villages.

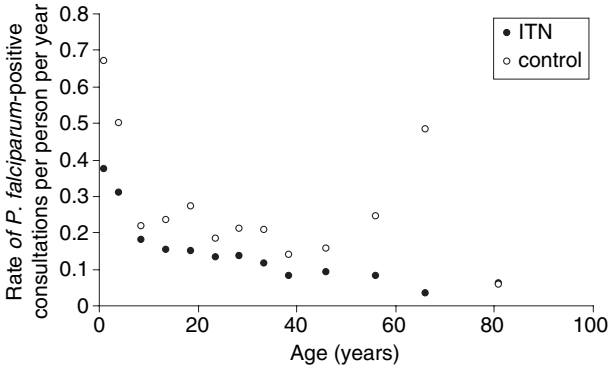

Figure 4 Age-specific *Plasmodium falciparum*-positive consultation rates per person per year in ITN and control villages during the study period (November 2001 to August 2002).

$P = 0.263$) in those aged over 5 years, this suggests a greater effect of ITN on *P. falciparum* incidence rates in younger children, although the effect is not significant in either age group.

Discussion

Although the estimates of ITN effect on *P. falciparum* incidence and prevalence in the total study population do not reach statistical significance, both indicate a protective effect (Table 5). The protective efficacies observed among the population of ITN when compared with control groups after adjusting for confounders were 9% (95% CI –28, 35%) for *P. falciparum* prevalence and 28% (95% CI–8, 53%) for *P. falciparum*-positive consultation rates, suggesting a greater reduction in overall *P. falciparum* incidence. The consistent protective direction of these estimates indicates a benefit of ITN, which the trial did not have the power to detect with statistical significance, although the possibility of no ITN effect cannot be ruled out. The original sample size calculation was found subsequently to have underestimated the power of the study, as it used villages as the sampling units and did not take into account their population size. In addition, the expected protective efficacy of 50% in the original power calculations was an overestimate. Rather than showing *post hoc* power calculations, we present the confidence intervals in the results that contain all the information on the power of the study (Smith & Bates 1992).

The 9% protective efficacy with respect to *P. falciparum* prevalence is similar to the meta-analysis of seven ITN trials in areas of stable malaria (annual EIR > 1) in Africa (Lengeler 2004) where the estimate was 13%. In an ITN trial in Thailand, the estimate was 20% (although this was non-statistically significant) (Luxemburger *et al.* 1994). At first sight, the 28% protective efficacy with respect to *P. falciparum* incidence observed in Cambodia appears small when compared with the 50% seen in the meta-analysis of four trials in Africa (Lengeler 2004) and the 41% in two trials in Thailand (Kamol-Ratanakul & Prasittisuk 1992; Luxemburger *et al.* 1994). However, three of the four African studies were conducted among children aged under 6 years, as were all the seven cited earlier in relation to prevalence measures. In our study, the protective effect of ITN in under 5-year-olds appeared to be considerably stronger than that in the rest of the population, with an estimated odds ratio for *P. falciparum* infection of 0.63 (95% CI 0.26–1.53) and relative reduction in the *P. falciparum*-positive consultation rate of 35%

Table 4 *Plasmodium falciparum*-positive consultation rates per person per year in matched village pairs. Rate ratios compare ITN with control villages

	Village population size		Number *P. falciparum*-positive consultations		Rate per person per year unadjusted		Rate per person per year age–sex adjusted			
Pair	ITN	Control	ITN	Control	ITN	Control	ITN	Control	Rate ratio age–sex adjusted	Rate ratio adjusted for age, sex, village population size, baseline prevalence
1	875	84	36	23	0.05	0.33	0.05	0.34	0.16	–*
2	711	255	43	31	0.07	0.15	0.08	0.16	0.48	
3	294	390	17	29	0.07	0.09	0.07	0.08	0.87	
4	193	593	53	17	0.33	0.03	0.33	0.03	9.67	
5	424	398	33	76	0.09	0.23	0.09	0.21	0.41	
6	191	215	33	58	0.21	0.32	0.21	0.34	0.62	
7	183	187	41	117	0.27	0.75	0.25	0.79	0.31	
8	743	215	91	84	0.15	0.47	0.13	0.48	0.28	
9	276	172	36	116	0.16	0.81	0.17	0.84	0.20	
10	223	354	86	137	0.46	0.46	0.43	0.48	0.89	
11	381	353	106	122	0.33	0.41	0.35	0.40	0.87	
12	301	245	69	109	0.28	0.53	0.25	0.60	0.42	
13	101	258	74	111	0.88	0.52	0.90	0.54	1.68	
14	257	505	78	89	0.36	0.21	0.39	0.20	1.92	
15	553	173	124	243	0.27	1.69	0.25	1.85	0.13	
16	187	143	43	32	0.28	0.27	0.29	0.29	1.03	
17	213	80	39	111	0.22	2.08	0.22	1.68	0.13	
Mean	359	272	59	89	0.21†	0.36†	0.20†	0.36†	0.56† (95% CI 0.32–0.99) $P = 0.047$	0.72† (95% CI 0.47–1.08) $P = 0.106$

* Overall rate ratio calculated only.
† Geometric mean.

Table 5 Summary of main results. Estimates compared ITN villages with control villages

Outcome	Analysis*	Estimate type	Whole population (95% CI)	*P* value	Children <5 years (95% CI)	*P* value
P. falciparum prevalence	*t*-Test, adjusted by the method of Bennett *et al.* 2002	Prevalence ratio	0.91 (0.65–1.28)	0.581	–	–
	Logistic regression GEE	Odds ratio	0.81 (0.38–1.74)	0.586	0.63 (0.26–1.53)	–**
P. falciparum-positive consultation rate (incidence proxy measure)	*t*-Test, adjusted by indirect standardization & log linear models	Rate ratio	0.72 (0.47–1.08)	0.106	0.65 (0.33–1.28)	0.197

* All analyses were matched and adjusted for age group, sex, baseline prevalence and village population size.
** *P* value for the interaction between ITN intervention status and being aged under/over 5 = 0.042.

(95% CI –28, 67%). The magnitude of ITN impact on *P. falciparum* incidence in this setting therefore appears to be about 70% of that observed in previous African trials. The increased benefit among young children may be because a higher proportion of infectious bites cause infection and disease in children. This is due to both a lower initial absolute number of bites, caused by both their smaller body mass and surface area (Port & Boreham 1980) and a less well-developed immunity. Additionally, they may go under the ITN earlier in the evening.

These estimates of ITN effect suggest that the differences in malaria epidemiology and vector behaviour in South East Asia when compared with that observed in African trials reduce the magnitude of ITN impact. Transmission

intensity in Cambodia is lower than that in any of the trials in Africa; the EIR being estimated at 6.0 (Trung *et al.* 2004), compared with EIRs of 10–300 or more in the African settings. Low transmission rates may increase the protective effect of ITN (Lengeler 2004). On the contrary, the habits of *A. dirus*, when compared with the major African vector *A. gambiae*, may act to reduce the protective effect of ITN. The earlier biting times of *A. dirus* of 2100–2400 may have been a contributing factor, although a sociological survey reported an average bedtime of 2100 h in the study villages (Brown *et al.* 2002). *Anopheles dirus*, like *A. gambiae*, is anthropophilic, and therefore the mass effect on mosquito population density sometimes observed in Africa at high levels of ITN coverage might be expected in Cambodia. However, the epidemiological analysis did not identify a mass effect and the intensive entomological surveys required to confirm such an effect were not feasible in this remote area.

There is also a question of whether the relative impact of the ITN intervention would be different had the antimalarial treatment been any different. The increase in *P. falciparum* incidence and prevalence with transmission intensity has been found to be non-linear, rising steeply at lower transmission intensities, then levelling off. This is thought to be because the proportion of bites, which cause new infections and clinical disease, decreases at higher transmission intensities (Beier *et al.* 1994; Charlwood *et al.* 1998; Smith *et al.* 2004). By reducing transmission with the artesunate combination therapy (ACT) intervention, it is possible that the further reduction caused by ITN was able to have greater impact on malaria prevalence and incidence. The effect of the ACT intervention may have led to bias, as the control villages had a higher rate of *P. falciparum*-positive consultations, and therefore ACT treatment, than intervention villages (0.36 *vs.* 0.21 per person per year). The use of ACTs almost certainly contributed to an ongoing reduction in transmission, as suggested by the before–after comparison of *P. falciparum* prevalence in control villages, which fell from 30% at the first cross-sectional survey to 18% at the second.

One limitation of this trial is that prevalence was the most accurately measured outcome rather than the incidence of disease episodes, which for the patient is more important than removing asymptomatic infections. As a measure of incidence, the *P. falciparum*-positive consultation rate has limited accuracy owing to unknown rates of recrudescence, the existence of other febrile diseases among asymptomatics or alternative treatment-seeking behaviour among malaria cases. The denominators for incidence calculations were also crude, as there was no information on population changes over time. The strong association between *P. falciparum*-positive consultation rate and baseline *P. falciparum* prevalence suggests that there is a relationship with true *P. falciparum* incidence. However, it was also strongly correlated with village population size, which is unlikely to be associated with true *P. falciparum* incidence in view of the lack of association between village population size and *P. falciparum* prevalence in the cross-sectional survey data ($P = 0.753$, analysis not shown). This correlation may instead reflect differences in treatment-seeking behaviour between small and large villages, because of, for example, easier access to the village malaria worker or less access to private drug sellers in smaller villages. Adjusting for village population size in this analysis has probably controlled for these related factors to some extent, but because the factors were not measured, there could be residual confounding if there were imbalances between trial arms. Another potential bias in the estimate of ITN effect on incidence arises because participants could not be blinded as to their intervention status. Inhabitants of control villages may have been more likely to believe they had malaria, increasing the consultation rate with the village malaria worker and detection of asymptomatic infections.

The reported ITN coverage in intervention villages was high (mean of 87%), but the reliability and consistency of reported use is uncertain. According to the sociological survey (Brown *et al.* 2002), the semi-nomadic lifestyle of the study population appeared not to affect ITN use, when families went to stay at their farms: 87% reported taking nets. In contrast, only 25% of people took ITN on overnight trips to the forest. This indicates potential for use of smaller individual 'hammock' nets that are more practical to transport. The sociological survey noted that nets were washed relatively often, mainly because of soiling by children, from once a month to as often as once a week. Recent findings are that deltamethrin-impregnated polyester nets are relatively resistant to washing, still causing 81.8% mortality in *Anopheles* after 12 washes and 64.8% after 15 washes (Graham *et al.* 2005). Nonetheless, more frequent insecticide impregnation or long-lasting insecticide-treated nets may be beneficial in such settings where washing is frequent. A small-scale entomological study that took place in June and July 2002 within the context of the trial suggested no functional change in insecticide sensitivity of vectors in the area. The median knockdown time for *A. dirus* samples exposed to deltamethrin was marginally lower in ITN than control villages but the mortality rate was not (Blonsky & Rowland 2002).

Villages were selected for inclusion in the trial on the basis of not previously having been provided with ITN under the national programme. They varied considerably both in terms of accessibility and in terms of malaria endemicity and were likely to have been generally

representative of rural North East Cambodia. This suggests that our findings will be relevant to much of forested South and South East Asia.

Cross-sectional surveys took place in September when traditionally the vast majority of people are back in their villages celebrating the harvest, minimizing any selection bias caused by the semi-nomadic lifestyle of the population. It should be noted that the household sampling method introduces further clustering that was not taken into account by the statistical analysis. Therefore, the width of the confidence intervals around estimates of ITN effect on prevalence may be underestimated.

Conclusions and recommendations

The lack of statistical significance of the results of this trial should not deter investment in ITN in South East Asia, as the trial was underpowered. The apparent impact on *P. falciparum* incidence of 28% relative reduction of disease episodes among the total population and 35% in children under 5 years of age justifies further investment in ITN. ITN may help reduce the current high child morbidity and mortality in parts of this region.

Further ITN studies in South East Asia would benefit from larger, more detailed entomological studies to evaluate the contribution of vector behaviour and transmission intensity to a possible mass effect. There is also a need to continue to monitor ITN use and maintenance.

Acknowledgements

We are grateful to the staff at the provincial and district health department offices in Rattanakiri Province who were involved in running the study, especially Nhem Hay, for all his hard work in collecting data and supervising the village malaria workers, despite extreme seasonal difficulties in accessing remote villages. We thank the survey teams from the National Center for Parasitology, Entomology and Malaria Control for their efforts during the prevalence surveys. We thank the village malaria workers for all their hard work and we thank the study participants for their cooperation. We are grateful to the staff at the project management unit of the EC-Cambodia Malaria Control Programme for their timely logistical support. Our thanks go to Seshu Babu and Esther Sedano for their helpful suggestions during the protocol development phase and for their technical inputs during the prevalence surveys.

We are also grateful to Allan Schapira, Stephan Hoyer and Reiko Tsuyuoka for their active support for the trial, to Paul Milligan and Andrew Thomson for advice on data analysis and to Jonathan Cox for providing the maps presented in Figure 1. This project received financial support from the European Commission-Cambodia Malaria Control Programme.

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Corresponding Author **Sean Hewitt**, Vector Borne Disease Control Consulting Ltd., Llandysul, UK. Tel.: +44 (0) 1559 371127; E-mail: sean.hewitt@vbdc-consulting.com

Moustiquaires imprégnées d'insecticide pour la prévention de la malaria à *Plasmodium falciparum* au Cambodge: Essai randomisé en grappe

OBJECTIFS Valider et quantifier l'impact des moustiquaires imprégnées d'insecticides sur l'infection de la malaria au Cambodge.

MÉTHODES Essai randomisé en grappe par paire basé sur l'utilisation de moustiquaires imprégnées d'insecticides à Rattanakiri dans le nord-est du Cambodge. 34 villages avec une population de 10726 habitants ont été randomisés pour recevoir des moustiquaires imprégnées de deltamethrine ou comme contrôles ne recevant pas de moustiquaires. Des études transversales ont mesuré la prévalence de *P. falciparum* au début et 10 mois après la distribution de moustiquaires. Des volontaires dans les villages, issues du groupe contrôle ou de celui recevant l'intervention ont été responsables du traitement à l'artésunate et à la méfloquine des cas testés positifs pour *P. falciparum* au moyen de test sur bandelettes. Les données de surveillance passive résultant de cette étude ont été utilisées pour estimer l'incidence des infections cliniques à *P. falciparum*.

RÉSULTATS Une efficacité de protection de 28% (OR ajustés = 0,72; IC95%: 0,47–1,08) sur l'incidence et 9% (OR ajustés = 0,91; IC95%: 0,65–1,28) sur la prévalence de *P. falciparum* dans le groupe d'intervention par rapport au contrôle a été enregistrée. Cependant, aucune des ces estimations n'étaient statistiquement significatives. Une analyse au niveau individuel a indiqué une réduction plus importante de la prévalence de *P. falciparum* chez les moins de 5 ans (OR ajustés = 0,63; IC95%: 0,26–1,53) que chez les plus âgés (P d'interaction = 0,042). L'efficacité de protection de 35% (IC95%: 28–67) sur l'incidence de *P. falciparum* clinique chez les moins de 5 ans était plus prononcée que celle sur l'estimation de prévalence correspondante, mais n'était pas significative.

CONCLUSION Il est probable que le manque de signification statistique dans les résultats soit dû au manque de poids des données. L'analyse révèle plus d'évidence sur l'efficacité des moustiquaires imprégnées d'insecticides dans le nord-est, surtout chez les moins de 5 ans.

mots clés insecticide, moustiquaires, malaria, *P. falciparum*, Cambodge, essai

Redes mosquiteras impregnadas para la prevención de malaria por *Plasmodium falciparum* en Camboya: ensayo aleatorizado por grupos

OBJETIVOS Validar y cuantificar el impacto de las redes mosquiteras impregnadas en la infección por malaria en Camboya.
MÉTODOS Ensayo aleatorizado, pareado y por grupos, de redes mosquiteras impregnadas en Rattanakiri, noreste de Camboya. En 34 poblados con una población total de 10,726 habitantes se aleatorizó para recibir mosquiteras impregnadas con deltametrina o control (sin red mosquitera.) Mediante croseccionales se midió la prevalencia de *P. falciparum* antes de comenzar el estudio y 10 meses después de la distribución de las redes. Los voluntarios en los poblados con intervención y de control trataron a los que daban positivos para *P. falciparum* mediante la prueba del *dipstick* con artesunato y mefloquina. Los datos obtenidos por detección pasiva de casos fueron utilizados para estimar la incidencia de infecciones clínicas de *P. falciparum*.
RESULTADOS Se encontró una eficacia protectora del 28% en la incidencia de *P. falciparum* (tasa ajustada 0.72, 95% IC 0.47–1.08) y 9% en la prevalencia de *P. falciparum* (tasa ajustada de prevalencia 0.91, 95% IC 0.65–1.28) en los poblados intervenidos vs. los poblados control; sin embargo, ninguno de estos estimativos era estadísticamente significativo. El análisis a nivel individual indicó una mayor reducción en la prevalencia de *P. falciparum* en menores de 5 años (OR ajustado = 0.63 95% IC 0.26–1.53) que en individuos mayores (interacción P = 0.042). La eficacia protectora del 35% (95% IC–28, 67%) con respecto a la incidencia clínica de *P. falciparum* en menores de 5 años fue más pronunciada que los correspondientes estimativos para prevalencia, pero de nuevo sin ser estadísticamente significativo.
CONCLUSIONES La falta de significancia estadística en los resultados puede deberse a una falta de poder estadístico. El análisis provee más evidencia de la efectividad de las redes mosquiteras impregnadas en el noreste de Camboya, particularmente en menores de 5 años.

palabras clave insecticida, redes mosquiteras, malaria, *P. falciparum*, Camboya, ensayo