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Entomological evaluation of community-wide use of lambdacyhalothrin-impregnated bed nets against malaria in a border area of north-west Thailand

Pradya Somboon[1], **Jo Lines**[2], **Apinun Aramrattana**[3], **Udom Chitprarop**[4], **Somsak Prajakwong**[4] and **Chirasak Khamboonruang**[5] [1]*Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand;* [2]*Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK;* [3]*Department of Family Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand;* [4]*Malaria Centre, Region 2, Chiang Mai 50200, Thailand;* [5]*Research Institute for Health Sciences, Chiang Mai University, Chiang Mai 50200, Thailand*

Abstract

This paper reports 2 studies. (i) After a year of baseline data collection, lambdacyhalothrin-treated bed nets were introduced into 3 of 5 villages in north-west Thailand, the remaining 2 being treated with placebo. Human bait collections were carried out in each village on 2 nights per month, for 8 months of each year, and the biting densities were compared between the first year and the second year. The treated bed nets did not have any significant impact on the density or parous rates of *Anopheles sawadwongporni* and *A. maculatus s.s.* populations. The results for *A. dirus s.l.* were not conclusive because of the low number caught. Significant reductions in biting and parous rates of *A. minimus* species A were observed in only one of the 3 treated villages, and there was no overall difference between treated and control groups. However, the trial suffered from the washing of nets by villagers and the low rate of reimpregnation. (ii) A short-term study involved 4 villages in a cross-over design, and lasted 48 d. For the first 24 d, residents of 2 villages were given new treated nets while the other 2 villages retained their own untreated nets. For the second 24 d, this situation was reversed. Daily light-trapping revealed no significant difference in the indoor densities or parous rates of *A. minimus* species A between the periods with treated or untreated nets. Both studies, especially the second, suggested that the community-wide use of treated bed nets did not generally reduce the vectorial capacity of vectors in this area, probably because of the biting behaviour of the mosquitoes

Keywords: insecticide-impregnated bed nets, *Anopheles sawadwongporni, A. maculatus, A. dirus, A. minimus*, biting rate, parous rate, Thailand

Introduction

Malaria is a major public health problem in Thailand, especially in forest and border areas. Control of malaria transmission by DDT house spraying, although successful in interrupting malaria transmission in most of the plains areas of the country, has experienced many problems elsewhere, including the exophilic behaviour of all main vector species (i.e., *Anopheles minimus s.l., A. dirus s.l.*, and *A. maculatus s.l.*), the increasingly poor acceptance by communities of the spraying, and the movement of people to intense foci of transmission both inside and outside the country (KETRANGSEE *et al.*, 1991).

One possible alternative method for control of malaria is the use of pyrethroid-treated bed nets (ROZENDAAL, 1989; WHO, 1989; CURTIS *et al.*, 1990), which have shown benefits in reducing the number of bites on humans sleeping within them, even if there are holes in them, or on humans nearby because of the deterrent and excito-repellent effects and feeding inhibition of the insecticide (LINES *et al.*, 1987). When used by a whole community, the treated nets might be expected to have a 'mass effect', reducing the density and longevity of vector populations, and thus providing an additional benefit above the improved personal protection provided by individual use. However, of the numerous trials reviewed by CURTIS (1992) or reported more recently (SAMARAWICKREMA *et al.*, 1992; BEACH *et al.*, 1993; DAS *et al.*, 1993; HII *et al.*, 1993; KARCH *et al.*, 1993; KERE *et al.*, 1993; LINDSAY *et al.*, 1993a, 1993b), many were not designed to allow detection of a mass effect, and in others no mass effect was seen. The clearest evidence for a mass effect has come from trials with anthropophilic and endophilic *A. gambiae* in Africa (MAGESA *et al.*, 1991; ROBERT & CARNEVALE, 1991; KARCH *et al.*, 1993), with endophilic populations of *A. minimus s.l.* in India (JANA-KARA *et al.*, in press), and with *A. farauti* in the Solomon Islands (KERE *et al.*, 1993). However, evidence for the absence of a mass effect with *A. gambiae s.l.* was reported from The Gambia; this may have been due to the mixing of mosquito populations between villages with treated and untreated bed nets (LINDSAY *et al.*, 1993b). In the *A. punctulatus* complex in Papua New Guinea, there was a reduction in the human blood index and a shift in the peak time of biting from late to early in the night, but no evidence for a reduction in density or longevity (MILLEN, 1986; CHARLWOOD & GRAVES, 1987; GRAVES *et al.*, 1987).

Starting in late 1989, a large-scale epidemiological trial of lambdacyhalothrin-treated bed nets was carried out in north-west Thailand. This paper presents the entomological evaluation, which consisted of a long-term and a short-term study. The impact on malaria incidence will be presented separately by A. Aramrattana *et al.* (paper in preparation).

Materials and Methods

The long-term evaluation

Study area. The study was carried out in Karen villages located in forest and forest fringe areas in Mae Sariang district, Mae Hong Son province, north-west Thailand, adjacent to the Myanmar border. The province is extremely mountainous and forested, with very limited plains areas. Forest fringe villages are typically situated in foothills surrounded by rice fields, scrub and secondary forest, with streams running through or alongside them. Farm huts, the temporary shelters to which villagers move during ploughing and harvesting periods, are scattered in rice fields in the valleys and on the mountains. Some villages are situated deeper in the hills and in the forest. Cattle and pigs are common in most Karen settlements.

Four forest fringe villages, Mae Han, Mae Chon, Mae Top Nua and Mae Salap, and a deep forest village, Huai Ngu, were selected for the study. These villages were among the 24 units of a parasitological evaluation (ARAMRATTANA, 1993). Mae Salap and Huai Ngu are isolated villages. At Mae Top Nua there is a neighbouring village about 500 m away, separated by 2 hills. Mae Han and Mae Chon are separated from each other by about 800 m and a hill. The annual malaria parasite incidence in 1989 varied from 80·7–279·7 per 1000 persons. Details of the population and bed net usage are given in Table 1. Detection of malaria sporozoites by enzyme-linked immunosorbent assay (BURKOT *et al.*, 1984; WIRTZ *et al.*, 1987) has suggested that *A. minimus* species A, *A. sawadwongporni* (formerly *A. maculatus* species A), *A. maculatus s.s.* and *A. dirus s.l.* are the potential vectors in the area (SOMBOON, 1993).

Table 1. Population and numbers of bed nets in the 5 study villages, Mae Han, Mae Top Nua, Mae Chon, Mae Salap, and Huai Ngu, in the long-term study

	Control villages		Treated villages		
	Mae Han	Mae Top Nua	Mae Chon	Mae Salap	Huai Ngu
No. of households	149	21	20	57	23
Population[a]	719	116	90	261	118
No. of nets currently used[b]	304	43	38	118	48
No. of persons/net	2·4	2·7	2·4	2·2	2·5
No. of nets treated					
First treatment[c]	55 (18·1%)	36 (83·7%)	31 (81·5%)	96 (81·4%)	47 (97·9%)
Second treatment[d]	18 (5·9%)	12 (27·9%)	14 (36·8%)	37 (31·4%)	30 (62·5%)

[a]Data from Mae Sariang Malaria Sector (1990).
[b]Surveyed in September–November 1990.
[c]February/March 1991.
[d]October/November 1991.

Since the coverage of DDT spraying in the study villages had been very poor for a number of years and there was little evidence that this method was still effective, spraying was suspended within a radius of 5 km from October 1989 until the end of the study. Other routine anti-malaria activities continued.

Study design. The study was divided into 2 phases, the first (baseline phase) during the high transmission season from May to December 1990 and the second (intervention phase) during the same months in 1991. In February/March 1991, bed nets in Mae Chon, Mae Salap and Huai Ngu were treated with lambdacyhalothrin (10 mg/m^2) while those in Mae Han and Mae Top Nua were treated with placebo. The design permitted pre- and post-intervention comparisons in each village, and contemporary comparisons between treated and untreated villages.

Net census, impregnation and distribution. Most people in the area used cotton or cotton–polyester nets. In an attempt to obtain a high rate of net usage, a census was carried out in early 1990 and a number of additional nets were distributed at a subsidized price. Over 80% of nets in all the entomological study villages were treated in the first round of impregnation, except for Mae Han where the coverage was very low (see Table 1). In the second round of treatment in October/ November 1991, relatively low coverage was found over the whole study area. Details of net impregnation and distribution will be described by A. Aramrattana *et al.* (paper in preparation).

Entomological data collection and analysis. Mosquitoes were collected on 2 human baits indoors and 2 outdoors for 2 nights each month from May to December 1990 (before intervention) and May to December 1991 (after intervention). The indoor collections were performed outside the sleeping room of villagers throughout the night, but the outdoor catches (5–10 m away) were made only until midnight. Similar collections were carried out simultaneously at the farm hut areas of each forest fringe village (located 2–3 km away). After morphological identification, the ovaries of mosquitoes were examined for parity (DETINOVA, 1962).

In each catching site each year, the monthly counts of mosquitoes, indoors plus outdoors, were transformed as log $(n+1)$ to stabilize the variance. Paired t tests were used to compare the difference between the mean log numbers before and after intervention on a monthly basis. Mantel-Haenszel χ^2 tests were used to compare the proportions of nulliparous and parous mosquitoes in each catching site, stratifying for corresponding months. EPISTAT® software (GUSTAFSON, 1989) was used to calculate and analyse data.

The short-term evaluation

Study design and study area. Since recruitment to mosquito populations fluctuates widely in the short term, more intensive measurements of mosquito density and parous rates on a daily basis may give a more reliable picture than less intensive occasional sampling over longer periods, as was done in the long-term study (CHARLWOOD *et al.*, 1985; CHARLWOOD & GRAVES, 1987; HOLMES & BIRLEY, 1987). Daily sampling increases the sample size in relation to variability so that small changes in the mosquito density and parous rate may be detected. A short-term study of the effect of treated bed nets on vector populations was therefore carried out in order to complement the long-term study. A cross-over design permitted each village to act as both control and treated units, thus allowing for the permanent effects of environmental differences. Washing of nets was unlikely to have taken place over the relatively short study period and was easy to check.

The study was conducted in 4 communities, 2 from the previous study (Mae Chon and Mae Top Nua), and 2 others: Mae Top Klang and the Wild Animal Conservation Centre. The human populations surveyed in May 1992 were 92, 99, 75 and 57, respectively. Mae Top Klang is another Karen village separated from Mae Top Nua by about 500 m and 2 hills (see above), and was involved in the previous epidemiological study as a control (untreated) unit in 1990–1991. The Wild Animal Conservation Centre is a government office with permanently resident staff living in Karen style houses. It is about 8 km north of Mae Top Nua, and surrounded by relatively dense forest.

Shortly before the study began, malaria workers had treated the bed nets in all the neighbouring villages as part of regional malaria control efforts. DDT spraying in these study sites had been suspended since October 1989 (see above).

Intervention method. House-to-house surveys were carried out one month before the intervention began. The purpose of the experiment was explained to the residents. Since Mae Top Nua and Mae Top Klang are close to each other, and there may have been some overlapping of their mosquito populations, they were treated in the same period. On 1 July 1992 new cotton–polyester bed nets treated individually with lambdacyhalothrin (10 mg/m^2) were distributed to everyone in Mae Top Nua and Mae Top Klang except the occupants of 3 sentinel rooms used for mosquito trapping. The bed nets of residents who already had them were temporarily stored to ensure that they used only the treated ones. On 25 July 1992 the treated nets were replaced with the occupants' own bed nets, which were untreated. In the other 2 communities, Mae Chon and the Wild Animal Conservation Centre, this pattern was reversed. At the end of the study all the treated bed nets were given free to the villagers. The villagers' bed nets were also treated as requested. In Mae Chon, where 36% of nets had been treated in late 1991 in the long-term study (about 6 months before this study began), people were encouraged to wash their nets before the study.

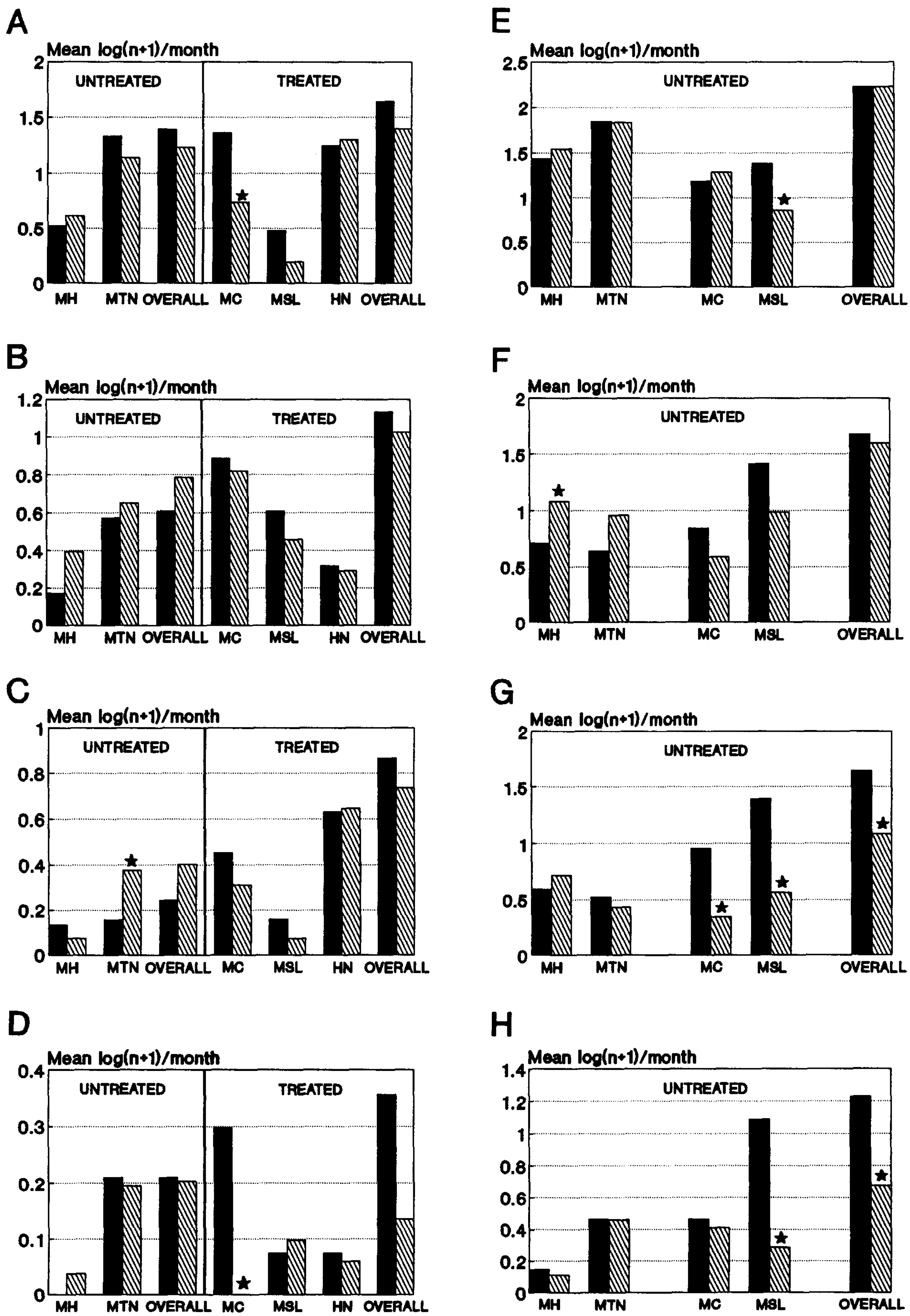

Fig. 1. Mean log $(n+1)$ numbers of mosquitoes per month collected on human bait, 2 indoors overnight and 2 outdoors until midnight, in the villages (A–D) and farm huts (E–H) of Mae Han (MH), Mae Top Nua (MTN), Mae Chon (MC), Mae Salap (MSL) and Huai Ngu (HN), before (May to December 1990, solid bars) and after (May to December 1991, hatched bars) intervention: A and E show figures for *A. minimus* species A, B and F for *A. sawadwongporni*, C and G for *A. maculatus s.s.*, and D and H for *A. dirus s.l.* Significant differences ($P<0.05$, paired t test) are indicated by asterisks (*).

Mosquito collection. CDC light-trap catches were used for daily collection of mosquitoes, hung beside occupied untreated bed nets (to avoid the deterrent and excito-repellent effects of the insecticide) in each of 3 houses in each community. They were connected with rechargeable batteries in the evening and disconnected in the morning by the project team. This method has shown a fairly good correlation with indoor human-biting catches in sampling *A. minimus* species A, which was the most abundant indoor biting species (ISMAIL *et al.*, 1982; SOMBOON, 1993). To reduce mortality of trapped mosquitoes caused by the wind from the trap's fan, the necks of the collection bags were extended to 35·5 cm, and the top of each bag was covered with a piece of wet cotton cloth. The mosquitoes collected from the 3 light traps in each community per night were pooled and identified to species. Most of them were dissected for parity.

The daily counts were log-transformed to reduce the variance. Comparisons of the geometric means between the periods when treated and untreated nets were used in each community were carried out by using Student's *t* tests. Analysis of variance (ANOVA) was carried out to partition variation into treatments and communities. The proportions of parous and nulliparous mosquitoes between the 2 periods were compared by using χ^2 tests.

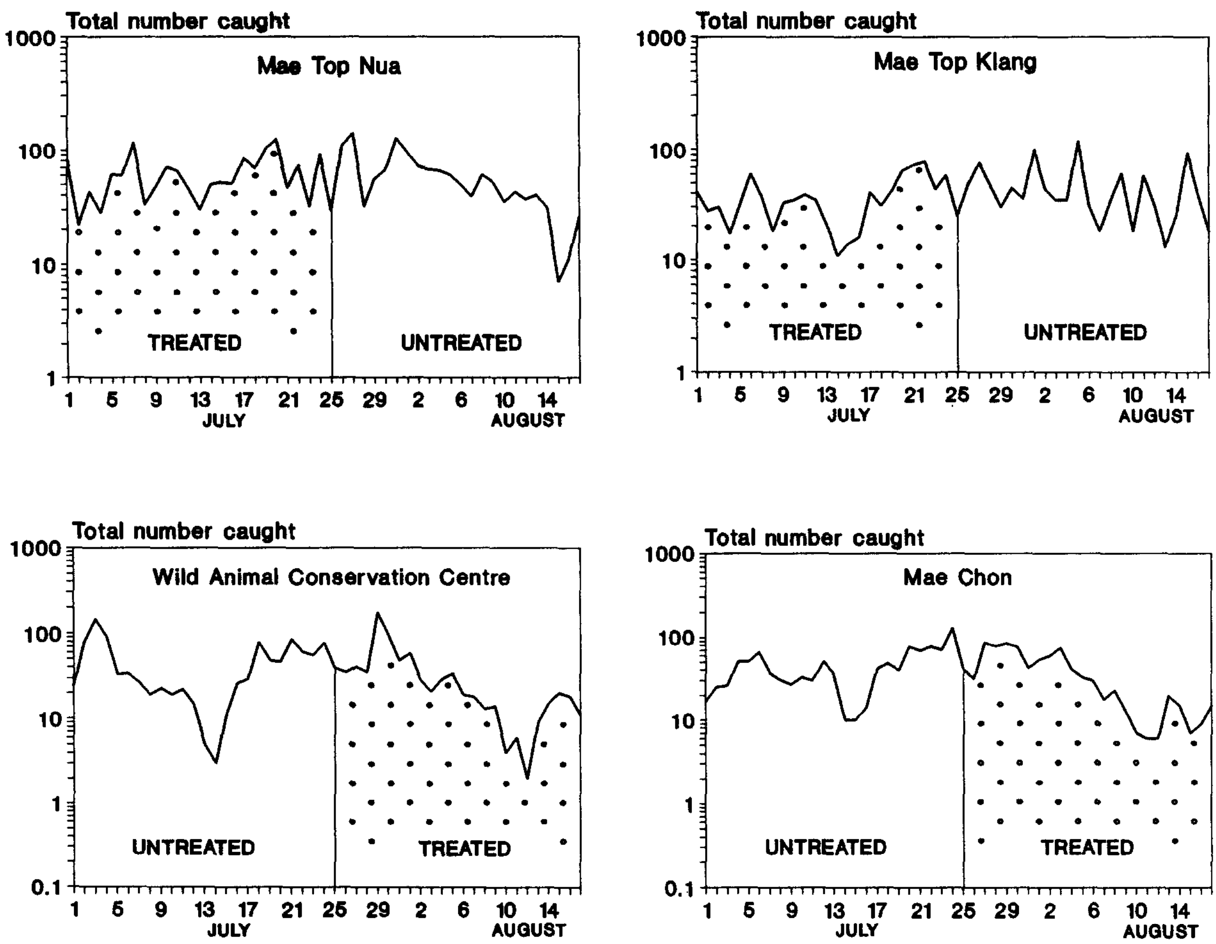

Fig. 2. Numbers of *A. minimus* species A females collected daily by 3 CDC light traps hung beside occupied untreated bed nets in houses in the periods when both treated and untreated nets were installed in 4 communities.

Table 2. Comparisons of the parous rates of *A. minimus* species A before (1990) and after (1991) intervention

	No. dissected		Parous rate (%)		Difference	
	1990	1991	1990	1991	(%)	χ^{2a}
Untreated villages						
Mae Han	35	65	57·1	56·9	−0·2	0·01
Mae Top Nua	196	352	67·3	73·6	+6·3	2·89
Overall	231	417	65·8	70·9	+5·1	2·71
Treated villages						
Mae Chon	307	102	68·4	53·9	−14·5	7·19*
Mae Salap	27	8	74·1	87·5	+13·4	0·10
Huai Ngu	180	294	62·8	70·4	+7·6	0·63
Overall	514	404	66·7	66·6	−0·1	0·76
Farm huts						
Mae Han	527	925	52·6	35·5	−17·1	30·10***
Mae Top Nua	748	718	47·9	54·7	+6·8	7·89å
Mae Chon	165	272	51·5	59·2	+7·7	0·32
Mae Salap	223	100	55·2	51·0	−4·2	0·93
Overall	1663	2015	50·7	46·3	−4·4	2·43

[a]Paired parous rates compared by Mantel-Haenszel χ^2 tests, stratifying for corresponding months; comparisons of overall parous rates were stratified by month and village. Significant differences are indicated thus: *$P<0·01$, ***$P<0·0001$.

Results

The long-term evaluation

Effect on mosquito densities. Mean monthly mosquito densities in 1990 and 1991 are shown by species in Fig. 1. From 1990 to 1991, a statistically significant reduction in the biting density of *A. minimus* species A was observed in the village collection in Mae Chon, but this effect was not seen in the other treated villages or in either untreated village.

There was no statistically significant change in the village biting densities of *A. sawadwongporni* and *A. maculatus s.s.* in any of the treated villages; a significant increase in *A. maculatus s.s.* biting rate was observed in the untreated village Mae Top Nua. The density of *A. dirus s.l.* was significantly decreased in Mae Chon, but not elsewhere.

When the numbers collected each month were pooled between villages in the treated and untreated groups, there was no significant change from 1990 to 1991 in either group.

In the farm hut areas, where no intervention was carried out, there were statistically significant changes (mostly decreases) in the densities of each of the 4 species in some sites. However, the overall densities of only *A. maculatus s.s.* and *A. dirus s.l.* were significantly reduced.

Effect on parous rates. Changes in parous rates were broadly similar to the changes in density. The results are shown in detail only for *A. minimus* species A (Table 2); those for the other species were similar. Although the total numbers caught were reasonably large, the sample size of a given species in a given village was usually quite small, and few of the year-on-year comparisons were significant. Overall, positive changes in parous rate outnumbered negative ones in treated as well as untreated villages. The only result that could possibly be interpreted as evidence for an effect of the nets was seen with *A. minimus* species A in Mae Chon, where there was a significant decrease in both density and parous rate. No calculation was made for *A. dirus s.l.* collected in the villages because of the very low numbers caught; at the farm huts, no significant change of the parous rate was observed.

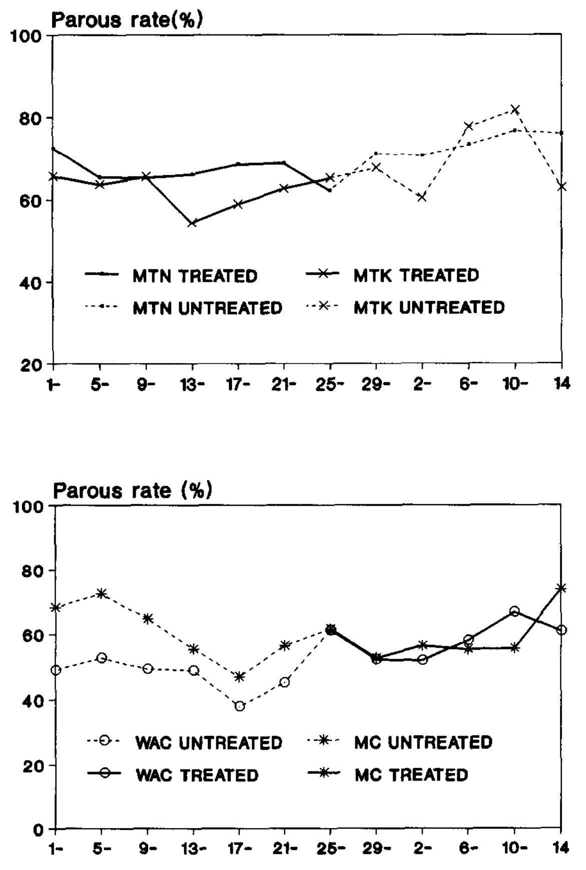

Fig. 3. Changes in the parous rate of *A. minimus* species A. Numbers of nulliparous and parous females collected in every four-night period were pooled. Broken lines show values when untreated bed nets were installed and the solid lines show those for treated bed nets. MTN=Mae Top Nua, MTK=Mae Top Klang, WAC=Wild Animal Conservation Centre, and MC=Mae Chon.

The short-term evaluation

Bednet usage. Excellent community participation was observed in all 4 communities. All residents used nets, except for a couple in Mae Top Nua who had never slept under a net and refused to use it. The proportion of residents who accepted the treated nets to replace their previous untreated nets ranged from 93% to 98%, excluding those in houses where light traps were used. Washing of a treated net was not observed during the study period.

Light trap catches. The numbers of *A. minimus* species A females collected each night from each community are shown in Fig. 2 and the patterns of the parous rates in Fig. 3. There were great fluctuations of both mosquito density and parous rate. Heavy rains followed by flooding occurred in the middle of the first period and at the end of July to early August. This seemed to be associated with a large reduction in the mosquito densities in most sites, which were observed 4–5 d after flooding.

Table 3. Analysis of variance on the log-transformed numbers of *A. minimus* species A collected by CDC light-traps hung beside occupied untreated bed nets in the periods when both treated and untreated were installed

Source of variation	Sum of squares	Degrees of freedom	Mean square	F	P
Community	2·2334	3	0·7444	7·31	0·0001
Treated net	0·2855	1	0·2855	2·81	0·096
Community× treated net	0·4678	3	0·1559	1·53	0·2
Residual	18·7378	184	0·1018	–	–
Total	21·7245	191	–	–	–

The ANOVA results (Table 3) showed that there was significant variation of *A. minimus* species A densities among the study communities. The effect of treated bed nets was of borderline significance (P=0·096). During the second half of the study period, there was a declining trend in densities in 3 of the 4 villages, including 2 of those with treated nets. However, if this had been caused, at least in those 2 villages, by the intervention of treated nets, there should have been a corresponding decline in parous rates. In fact, there was a slight increasing trend, suggesting that the decline in density was related to a decline in recruitment.

Discussion

The 2 studies described here differed not only in their timing but also in the intensity of entomological monitoring. The long-term study, which covered 8 months of the year, yielded adequate numbers of *A. sawadwongporni* and *A. maculatus s.s.* as well as *A. minimus* species A, but suffered from the washing of about 40% of the nets by villagers after the first impregnation, and from the low rate of reimpregnation. These problems were probably associated with the net washing habit of villagers, side-effects of the insecticide and the timing of reimpregnation during the period of harvest, when most people were in the rice fields all day. The short-term study was more focused and did not suffer from the problems of net washing and low coverage. Washing nets is known to reduce greatly the insecticidal activity of treated nets (MILLER *et al.*, 1991).

When the samples collected in the long-term study were compared between 1990 (pre-intervention) and 1991 (post-intervention), for each species and village, a broadly similar pattern was seen in both densities and parous rates. The great majority of year-on-year comparisons showed no significant change, and there was no hint that densities and parous rates in treated villages were more likely to decrease, or less likely to increase, than in untreated villages. Presumably, therefore, the significant changes that were seen were due to environmental factors rather than the intervention.

The fact that there was no apparent impact of the nets on populations of *A. sawadwongporni* and *A. maculatus s.s.* is not surprising. These 2 species are largely exophagic and zoophilic (UPATHAM *et al.*, 1988), and would not be expected to be greatly affected, at the population level, by the introduction of treated nets, even in conditions of high coverage. Neither was it very surprising that there was no consistent evidence for an effect of treated nets on any species in the farm hut setting. Although some villagers did take their treated nets with them on visits to the farm huts, many did not, and the proportion of potential hosts under treated nets was always much lower at the farm huts than in the villages.

One might have expected greater impact on the more anthropophilic *A. minimus* species A and *A. dirus s.l.*, but there was little evidence for this. The strongest evidence came from the village Mae Chon; in the long-term study, the densities and/or parous rates of these species were significantly lower in 1991 than 1990. Was this because the nets did have an effect in Mae Chon, but for some reason did not do so in Mae Salap and Huai Ngu, or were the decreases in Mae Chon merely a reflection of local environmental changes? There can be no conclusive answer to this question, but evidence in favour of the latter explanation, in the case of *A. minimus* species A, is available from the short-term study.

In the short-term study, densities of *A. minimus* species A in Mae Chon and in the Wild Animal Conservation Centre did decrease in the second half of the study, following the introduction of treated nets. However, there are 3 reasons for believing that this was not due to the treated nets. Firstly, a very similar pattern was seen at the same time in Mae Top Nua, where treated nets had been in use during the first half of the study and had just been removed. Secondly, the decrease in density was accompanied by a slight increase in parous rates, as would be expected if it were due to a decrease in recruitment, and the opposite of what would be expected if it were due to an increase in mortality. Thirdly, the decline in numbers in both Mae Chon and the Wild Animal Conservation Centre began 6–10 d after the introduction of treated nets, and continued for another 10–12 d. This is not the pattern that would be expected if it were an effect of treated nets. Assuming that the parous rates observed in this study (50–70%) are a reasonable guide to rates of survival per gonotrophic cycle, few of the mosquitoes alive on a given day can be expected to remain alive one week later. Any killing effect by treated nets should therefore be manifest within a few days and be very close to its maximum within 10 d of intervention.

For these reasons, we conclude that the treated nets did not have a mass killing effect on *A. minimus* species A. The results agree with 2 other studies in Tak province, about 200 km south of Mae Sariang, by C. Green (unpublished data) and M. Prasittisuk (personal communication).

The absence of a detectable mass killing effect of treated nets on *A. minimus* species A contrasts with the results of JANA-KARA *et al.* (in press) in Assam, India. This is presumably related to the biting behaviour of mosquitoes. *A. minimus* species A in Thailand is exophagic, exophilic, and readily bites humans and animals throughout the night (ISMAIL *et al.*, 1974; RATANATHAM *et al.*, 1988; P. Somboon, unpublished data). *A. minimus* in Assam, by contrast, is endophagic, endophilic and anthropophilic with a biting peak after midnight (RAO, 1984; JANA-KARA *et al.*, in press); similar behaviour used to be observed in Thailand before extensive DDT house spraying. The morphological features of *A. minimus* in Assam (RAO, 1984) and *A. minimus* species A in Thailand (SUCHARIT *et al.*, 1988) are similar.

In addition, the existence of domestic animals in a human residential area can diminish the mosquito attack rate on humans (COLUZZI, 1984; BURKOT *et al.*, 1989). In most Karen communities, cattle are very common and can provide alternative blood meals. They are normally kept under a shelter away from houses. Blood meal identification of blood-fed *A. minimus* species A females collected by indoor light traps showed that about 50% of them ($n=89$) were from animal sources (P. Somboon, unpublished data).

The numbers of *A. dirus s.l.* were too low to permit a conclusive result. However, C. Green (unpublished data) observed in a small trial in Tak province that treated nets had a significant impact on *A. dirus* species A, but not species D. Further investigation on this species group is needed.

In conclusion, these results indicate that community-wide use of pyrethroid-treated nets does not generally reduce the vectorial capacity of populations of *A. minimus* species A and the *A. maculatus* group in north-west Thailand. This does not mean that treated nets are ineffective in protecting against malaria; an individual sleeping under a treated net should nevertheless gain good personal protection against vector biting. However, the lack of a mass effect does mean that there is no protection for people outside nets. From the point of view of an individual who does sleep under a treated net, it does not matter whether or not others in the same community do so as well. From the public health point of view, it means that there is no advantage (in terms of efficacy) in organizing the impregnation of nets on a community-by-community basis, rather than household-by-household, which may often be easier in practice.

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