THE IMPACT OF PERMETHRIN IMPREGNATED BEDNETS ON THE MALARIA VECTOR *ANOPHELES MACULATUS* (DIPTERA: CULICIDAE) IN ABORIGINAL VILLAGES OF POS BETAU PAHANG, MALAYSIA

I Vythilingam, LC Foo, GL Chiang, ST Chan, KL Eng, S Mahadevan, JW Mak and K Inder Singh

Institute for Meical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia

Abstract. The effect of permethrin impregnated bednets on *Anopheles maculatus* Theobald was studied in four villages in Pos Betau, Pahang, Malaysia from August 1990 to July 1992. Collections of mosquitos were carried out indoors and outdoors from 1900 to 0700 hours. All mosquitos were dissected for sporozoites and parity. In May 1991 two villages received bednets impregnated with permethrin at 0.5 g/m^2 and two villages received placebo bednets. There was a significant difference in the sporozoite and parous rates between the treated and control villages after the distribution of bednets ($p < 0.05$). There was no significant difference in the bites/man/night of *An. maculatus* between the pre and post treatment periods in the control villages. However there was a significant difference in bites/man/night between pre and post treatment in the treated villages ($p < 0.001$).

INTRODUCTION

Malaria continues to be a public health problem in Malaysia despite many cycles of DDT house spraying. Bednets have long been used by people for protection against mosquitos and malaria (Port and Boreham, 1982; Bradley *et al*, 1986; Lindsay and Gibson, 1988). However, bednets have serious shortfalls. For example, if the nets are torn or not properly tucked in, mosquitos get inside and bite people sleeping underneath. When any part of the body remains in contact with the net, the mosquitos can probe through the mesh and bite the person. To overcome these problems, insecticide impregnated bednets are being used increasingly to interrupt malaria transmission (Rozendaal, 1989; Curtis, 1989). Thus insecticide impregnated bednets, even if torn, should kill large numbers of mosquitos which contact the nets and thereby would reduce the vectorial capacity of the vector population (Curtis *et al*, 1990).

Studies undertaken in various parts of the world have shown that permethrin impregnated nets have caused marked reductions in the number of *Anopheles* biting man (Charlwood and Graves 1987; Lindsay *et al*, 1991; Magesa *et al*, 1991; Samarawickrema *et al*, 1992), with up to 70% reduction of malaria morbidity and mortality (Alonso *et al*, 1990). Therefore a study was conducted in Malaysia to evaluate the impact of permethrin impregnated bednets on *An. maculatus* Theobald, the main vector of malaria in this country.

MATERIALS AND METHODS

Study area

The study was carried out in Pos Betau, Pahang, Malaysia, an Aboriginal resettlement area in secondary jungle, about 150 kilometers east of Kuala Lumpur. The area has 17 villages mainly riverine, with a population of just over 1,000 people.

The houses in this area are mainly built of wood and have zinc roofs; most houses have an annexe built of bamboo with a thatched roof. The inhabitants usually sleep in these annexes. All houses had been sprayed repeatedly with DDT 2 g/m^2 at six monthly intervals, but this was suspended one year previously.

Our study began in August 1990 and ended in July 1992. Kuala Milot, Sat Baru, Meter and Kernip were chosen as indicator villages for entomological monitoring.

Mosquito collections

In the indicator villages 12 hour mosquito collections were carried out monthly, overnight from 1900 to 0700 hours with two collectors stationed indoors and two outdoors simultaneously. All mosquitos landing on human bait were caught using small tubes which were subsequently plugged with cotton wool. Two houses were selected in each village and two nights of collections were carried out

each month.

All mosquitos collected were counted and brought back alive to the laboratory the next day, identified and dissected to determine their parity and the malaria sporozoite rate.

Treatment of nets

Nylon 40 denier nets (from Bangla Klamboe, Siamdutch Mosquito Netting Co Ltd, Bangkok; a mixture of the small, medium and large sizes) were treated with permethrin (25% EC formulation, 40:60 cis : trans, ICI. "Imperator") diluted to deposit 0.5 g active ingredient per square meter, following procedures described by Schreck and Self (1985). Enough insecticide was mixed to treat 20 nets each time. After dipping each net, the excess insecticide was allowed to drain off and saved for re-use. Nets then were placed on plastic sheets in the shade to dry. Placebo nets were treated with mineral oil to give a white fluid resembling permethrin emulsion. Kuala Milot and Sat Baru were given treated nets whereas Meter and Kernip were control villages with placebo nets. Villagers were told not to wash the nets until further instructions from us. The community was involved in the treatment of nets, first in May 1991 and retreatment of nets took place in November 1991. Before the distribution of nets, a survey was carried out to determine the sleeping habits in each household so that adequate numbers and sizes of nets were distributed to augment those already used by 40% of the people. All nets were given code numbers.

Assays and data analysis

The insecticidal effect of the residual permethrin was evaluated by contact bioassay. A cuboid 6 cm metal frame was enclosed in a portion of the net and *An. maculatus* (laboratory reared, sugar fed females of the IMR standard susceptiable strain) were introduced into it and exposed for 10 minutes. After exposure, the mosquitos were removed to paper cups and their mortality recorded after 24 hours. The test was replicated three times for each net.

The permethrin content in the bednets was determined using gas -liquid chromatography (GLC) on net samples (n = 21) chosen at random immediately after treatment and four months later.

Statistical analysis

Analysis of the data was performed on a Vax 8350 computer using SAS program to check for significant difference in the mean mosquito bites per man night and the malaria sporozoite rates by the Wilcoxon two sample test.

Parasitological survey

Mass blood surveys were also carried out bi-monthly and all malaria positive cases were treated. The results will be reported elsewhere.

RESULTS

Before the introduction of bednets the mean malaria sporozoite rate in *An. maculatus* collected during August 1990 - April 1991 from treated villages (1.24) and control villages (0.5) was not significantly different. During the 14 months of post treatment monitoring, after the introduction of treated bednets in May 1991, the mean sporozoite rate for treated villages (0.02%) was significantly lower ($p < 0.05$) than in control villages (0.6%).

Biting densities of *An. maculatus* in the treated villages were also significantly lower after the introduction of bednets ($p < 0.001$), whereas in the untreated comparison villages there was no significant difference in the mean number of mosquito bites/man/night before and after intervention ($p > 0.05$).

In the intervention villages, before the introduction of bednets, 62% of the *An. maculatus* females dissected were parous whereas, after the introduction of treated bednets the parous rate was significantly reduced to 44.1% ($p < 0.001$). In the untreated comparison villages, the mean parous rate was 55.9% before and 52.4% after the introduction of bednets, not significantly different ($p > 0.05$).

The biting cycle of *An. maculatus* indoors before the introduction of the bednets showed two peaks: at 2230 - 2330 and 0230 hours. After the introduction of bednets the second peak was suppressed and the number of bites/man/night was more evenly distributed throughout the night. Biting activity remained unchanged outdoors during pre- and post- treatment periods (Fig 1) and continued as before both indoors

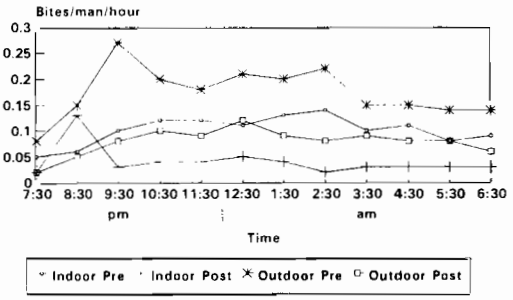

Fig 1–Biting activity of *An. maculatus* in villages with treated bednets.

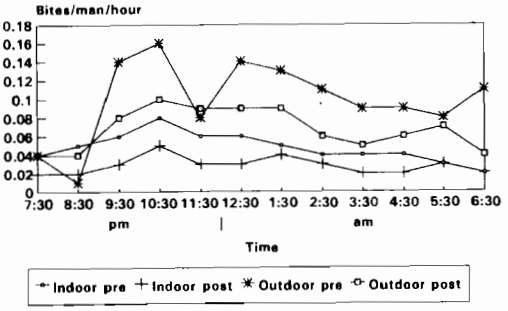

Fig 2–Biting activity of *An. maculatus* in village with placebo bednets.

and outdoors in the untreated comparison villages (Fig 2).

Bioassays on the nets showed that only 80% mortality was obtained from the treated nets one month after first impregnation in May 1991 and subsequently the mortality fell to below 60% after 6 months. In November 1991 the bednets were washed and retreated with permethrin. In subsequent bioassays at least 90% mortality was obtained even after 8 months of treatment.

Squares of bednets impregnated at the target dose of 0.5 g/m^2 were analysed by gas-liquid chromatography. Soon after they were treated the average permethrin level was 0.49 g/m^2 (range 0.36 - 0.61 g/m^2) and four months post- treatment was 0.23 g/m^2 (range 0.21 - 0.29 g/m^2).

DISCUSSION

In Sabah, Malaysia, Hii *et al* (1987) showed that there was a brief suppression of malaria incidence when insecticide treated bednets were used (in conjunction with radical drug treatment). In this study the number of *An. maculatus* bites per man-night were reduced when treated bednets were introduced to the villages. Lindsay *et al* (1989) also demonstrated that permethrin treated bednets used throughout a hamlet dramatically reduced the biting rate of malaria vectors in Africa.

The main advantage of pyrethroid treated bednets over DDT spraying is that when mosquitos alight on the nets they will try to squeeze through the netting to feed on the host. During this time the mosquitos can be expected to pick up a lethal dose of the insecticide. It has also been observed that with DDT spraying the

Table 1

Mean (and range) number of *An. maculatus* bites/man/night and malaria sporozoite rates, before and after installation of permethrin treated bednets, in the intervention and control villages.

	Pre-treatment		Post-treatment	
	Treated intervention	Control untreated	Treated intervention	Control untreated
Bites/man/night	20.3[a] (1.5-134.5)	11 (7-76.5)	7.9[a] (0-36)	7 (0-29)
Sporozoite rate	1.24[b] (0-33.5)	0.5 (0-8.3)	[b]0.02[c] (0-2.7)	0.6[c] (0-20)

Significance of difference:
[a]$p < 0.001$; [b]$p < 0.0001$; [c]$p < 0.05$ by the Wilcoxon two sample test.

mosquitos have developed behavioral resistance. They fly into houses, bite man and fly out (Hii and Chin, 1979).

Loong *et al* (1988) established that *An. maculatus* bites both indoors and outdoors. If mosquitos are deterred from houses with permethrin treated bednets and then bite outside, perhaps repeatedly, it might promote transmission, despite reduction in the density of indoor resting mosquitos.

In our study it was found that *An. maculatus* shifted to bite earlier in the night after the introduction of the bednets. With other species of malaria vectors a shift to an earlier biting cycle has also been reported by other workers (Charlwood and Graves, 1987; Magesa *et al*, 1993). This may be due to the way that mosquitos returning from oviposition were deterred from blood feeding on the same night because of the presence of the nets, and therefore delayed their next blood meal until early the following night.

There was a significant reduction in the malaria sporozoite rate between treated and control villages after the introduction of the bednets. In the treated villages mosquito collections were carried out seven months before the introduction of bednets and sporozoites were always present. After the distribution of treated nets, mosquito collections were carried out seven months and sporozoites were found on only one occasion. Thus it can be said that the permethrin impregnated bednets had a marked impact on entomolgical measures of malaria transmission. The simultaneous decrease in the number of parous female mosquitos in treated bednets villages, show that older, potentially infective, *An. maculatus* females were presumably being killed by permethrin.

From the gas-liquid chromatography it was observed that the permethrin content in nets varied, presumably due to the way that many people were participating in the treatment of the nets. It is important for the community to be involved if this strategy of using impregnated bednets is to be introduced to the malaria control program. Our results suggest that it would be sufficient to treat the bednets once in six months.

The people living in these villages are mobile, often spending months in the rainforest away from their homes. Thus, in theory it would be advantageous to distribute impregnated bednets to such a population to encourage them to take the bednets with them for use while in the forest, although in practice compliance is poor. Conversely, even if DDT residual spraying is to be carried out, it would not serve its purpose well, since the people so often sleep away from home.

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