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EVALUATION OF PERMETHRIN-TREATED MILITARY UNIFORMS FOR PERSONAL PROTECTION AGAINST MALARIA IN NORTHEASTERN THAILAND[1]

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ABSTRACT. A trial to compare the effect of military clothing treated by high-pressure spray with permethrin or placebo on the incidence of malaria in Royal Thai Army troops was conducted in northeastern Thailand. Bioassays of treated clothing using laboratory-reared *Anopheles dirus* females showed permethrin remained in the treated fabric for up to 90 days. Both permethrin- and placebo-treated uniform shirts provided >84% protection from biting *An. dirus* in laboratory bioassays for the duration of the study. In laboratory tests, knockdown of *An. dirus* exposed to permethrin-treated cloth fell to <20% after 3 hand washes, despite the presence of 28.7–59.9% of the original dose of permethrin. The use of permethrin-treated uniforms without adjunct application of topical repellents did not reduce malaria in Thai troops in an operational setting where incidence during 6 months was as high as 412 cases/1,000 in spite of chemoprophylaxis and use of untreated bednets.

INTRODUCTION

Malaria is a serious threat to Royal Thai Army (RTA) troops who routinely work along Thailand's borders with Cambodia and Laos. These remote, forested areas are endemic for *Plasmodium falciparum* and *Plasmodium vivax,* transmitted principally by *Anopheles dirus* Peyton and Harrison and *Anopheles minimus* Theobald (Prasittisuk 1985, Rosenberg et al. 1990). Conventional methods of vector control (e.g., insecticide spraying, source reduction) are difficult because of dispersed larval habitats, a lack of resources for the application of insecticide, and the large areas routinely traversed by the soldiers. Although consistently administered by the RTA, chemoprophylaxis against *P. falciparum* is largely ineffective, as the area is the center for multidrug-resistant *P. falciparum* in Southeast Asia (Looareesuwan et al. 1992). Since chloroquine-resistant *P. falciparum* was first reported in the area in 1962 (Harinasuta et al. 1962), this parasite has developed resistance to several antimalarial drugs and drug combinations. Fansidar/chloroquine is routinely used for chemoprophylaxis against malaria by the RTA, although this combination was found to be ineffective against *P. falciparum* in northeastern Thailand (Eamsila et al. 1993). Methods of personal protection to reduce man–vector contact in combination with effective chemoprophylaxis are therefore needed for prevention of *P. falciparum* malaria.

Recent studies have shown that the application of insecticides, primarily permethrin, to military clothing reduces the effects of biting mosquitoes (Gupta et al. 1987, Harbach et al. 1990), ticks (Evans et al. 1990), and chiggers (Frances et al. 1992). The use of permethrin-impregnated clothing, in combination with topically applied repellents containing diethyl methylbenzamide (deet) provided the wearer with good protection from biting arthropods. In some instances, the wearing of permethrin-treated uniforms alone provided better protection from mosquitoes than untreated uniforms, but this protection was less than that provided by a combination of permethrin-treated uniforms and topically applied deet. In tests conducted in Florida, Schreck et al. (1984) reported permethrin-treated clothing alone provided 89.1% protection from *Aedes taeniorhynchus* (Wied.) compared with 94.4% protection provided by the combination. Lillie et al. (1988) showed that permethrin-treated clothing reduced biting of *Culiseta impatiens* (Walker) by 93%, compared with 99% protection for the combination in tests in Alaska. Although these recent studies have shown that contact between humans and biting arthropods can be reduced by wearing permethrin-treated clothing, none of them have investigated the effects of such personal protection measures on the incidence of disease.

In this article we report a study to evaluate the effect of treating uniforms with permethrin on the incidence of malaria in RTA troops living

[1] The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense. Mention of a commercial product does not constitute an endorsement of the product by the Department of Defense. The volunteers gave informed consent to participate in the study.

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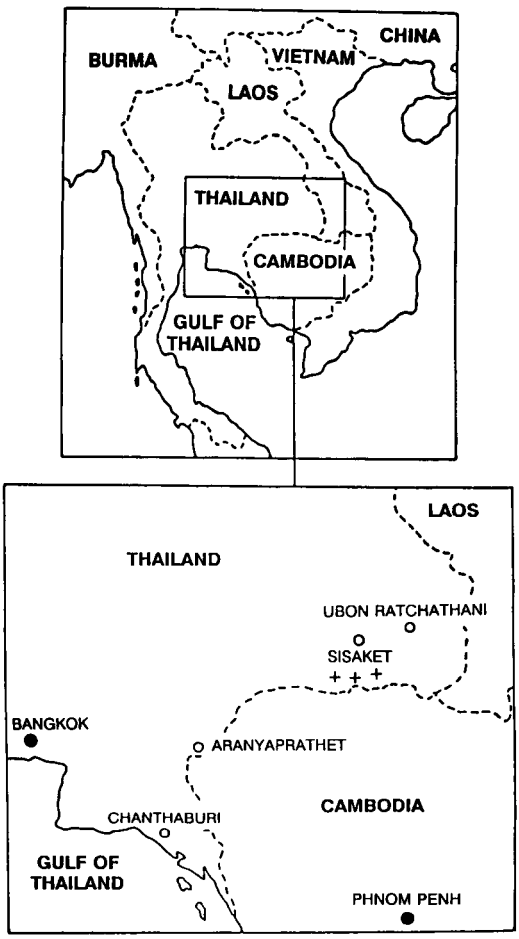

Fig. 1. Map of northeastern Thailand showing the location of study areas (+) in Sisaket Province.

and working in an area endemic for drug-resistant *P. falciparum* malaria in northeastern Thailand. Previous laboratory studies have shown that permethrin is lost from fabric following washing and wear (Schreck et al. 1980, 1982), so we also investigated the persistence of permethrin in clothing following treatment.

MATERIALS AND METHODS

Study sites and volunteers: Thai troops deployed to 3 areas near the Thai–Cambodian border in Sisaket Province, northeastern Thailand (Fig. 1) participated in this study between September 1991 and October 1992. In September 1991, 403 soldiers were recruited for these trials. Following a 6-month deployment some volunteers left the area and were replaced by new recruits. In April 1992, a total of 260 volunteers were participating in the study. The volunteers lived in locations (east, central, or west) approximately 20 km apart, 80 km south of the town of Sisaket (15°07′N, 104°20′E). These camps were not in heavily forested areas, but the volunteers often entered such areas during routine patrol duties. Most were recently inducted troops, aged 18–40 years, who had just finished basic training. Being from malaria-free areas of the country, they had acquired little or no immunity. During their deployment, all volunteers received Fansidar (sulfadoxine 500 mg plus pyrimethamine 25 mg) and chloroquine (500 mg) weekly. Prophylaxis was administered through paramedics and it was felt that compliance was generally good. In addition, bednets (not treated with permethrin) were available to all volunteers for use nightly.

Clothing treatment: An emulsion consisting of 151 ml of permethrin (as a 40% EC, U.S. National Stock Number 6840-01-334-2666, Coulston Products Incorporated, Avon, NY) and 7.5 liters of water were prepared in a 2-gal hand-pumped sprayer. Up to 4 uniform ensembles, consisting of shirt and trousers, were provided by each volunteer for treatment. Uniforms were laid on a large piece of plastic and the upper surface sprayed evenly at high pressure (approx. 55 psi), then they were turned and the lower surface sprayed until the entire uniform was saturated. Uniforms were then hung for up to 3 h to air dry. Using this method, 10–12 uniform ensembles could be treated with each 2 gal of formulation. Placebo uniforms were treated in the same manner, but a formulation consisting of 151 ml of kerosene and Tween 20 (Sigma Chemical Co.) as a surfactant in 7.5 liters of water was applied. Between September 23 and 25, 1991, the uniforms of 137 volunteers were treated with permethrin and those of 266 were treated with placebo. Between April 17 and 19, 1992, the uniforms of 112 volunteers were retreated with permethrin and those of 148 were retreated with placebo.

Monitoring procedures: Blood samples (thick and thin films) were obtained from each volunteer during a predeployment physical examination in September 1991, then subsequently collected during the last week of each month of the study, stained with Giemsa, and examined for malarial parasites. Because the deployments were not normally accessible to physicians, any sick soldiers reporting with a fever had blood smears taken by a paramedic. The number of cases of malaria was recorded, and grouped into 2 6-month periods (October 1991–April 1992 and April 1992–October 1992) for statistical

analysis using the chi-square test of significance.

Field bioassay procedures: To measure the insecticidal activity of the permethrin- and placebo-treated uniforms, shirts were obtained from the troops at various intervals after the April 1992 treatment, and subjected to a contact and biting bioassay using laboratory-reared 7–10-day-old *An. dirus* nulliparous females. Up to 9 permethrin- and 7 placebo-treated shirts were obtained from volunteers in the central location 1, 55, 90, and 180 days after treatment. Following each round of testing the shirts were returned to the volunteers.

A method developed by Schreck et al. (1980, 1982) was used to expose mosquitoes by tarsal contact to fabric and to record knockdown. Briefly, 7–10 test mosquitoes were placed between a plastic Petri dish lid (9.5 cm diam × 0.7 cm high) and a card (13 cm × 13 cm). A 1-cm² piece of dental dam with a small slit cut in it was attached to a similar-sized hole in the card to allow mosquitoes to be readily aspirated into the dish. The card-covered Petri dish was then placed onto the test fabric and the card removed to allow the mosquitoes to stand on the fabric. After 10-min exposure, the card was placed under the dish and the mosquitoes transferred into a 568-ml (1-pint) wax paper cup by placing the dish/card over the mouth of the cup and removing the card. The plastic Petri dish was used to cover the mouth of the paper cup. One hour after exposure, the mosquitoes were observed for knockdown. A mosquito was scored as knocked down if it was laying on its back or side, and was unable to maintain flight following a gentle tap on the cup. Two batches of mosquitoes were exposed to a similar place on the back of each shirt.

The biting bioassay involved placing 7–10 *An. dirus* females between a Petri dish base (9.5 cm diam × 1 cm high) and card with dental dam insert. For each biting test, a test shirt was held tightly over the thigh of one of 2 male volunteers (1 Thai and 1 Caucasian), and then the card was removed to expose the mosquitoes to the shirt for 5 min. The card was then returned and the mosquitoes liberated into a 568-ml (1-pint) wax paper cup. Sixty minutes later the number of bloodfed mosquitoes and knockdown was recorded. The test was then repeated with the second volunteer. The biting response of a sample of test mosquitoes was also assessed by exposing them to the bare thigh of each volunteer.

Persistence of permethrin following washing: Locally purchased fabric swatches (cotton–polyester blend in a camouflage pattern, light green heavy cotton twill, and dark green heavy cotton twill), all commonly used to make RTA field uniforms, were used for the tests. Three swatches (140 × 45 cm) of each fabric type were treated with permethrin using high-pressure spray apparatus in the field on April 18, 1992, air dried, wrapped individually in aluminum foil, and returned to our laboratory in Bangkok. There, 2 swatches of each fabric type were subjected to hand washing to simulate field laundry methods used by Thai soldiers. Fabrics were soaked in cool water (26°C) for 5 min, drained, then hand washed for 2 min in cool water containing 4 g/liter of household detergent. Fabrics were then rinsed twice in 4 liters of water and gently wrung out before air drying. Following each wash the cloth samples were subjected to bioassay using 4–5-day-old *An. dirus* nulliparous females exposed for 10 min using the method described above. Batches of mosquitoes were also exposed to untreated cloth, and to an unwashed sample of permethrin-treated fabric. Subsamples of the fabrics were obtained after 0, 3, and 6 hand washes for chemical analysis using high performance liquid chromatography apparatus. Subsamples approximately 10 cm² were cut from each type of fabric, wrapped individually in aluminum foil, and posted to the Australian Army Malaria Research Unit, Ingleburn, Australia, for analysis.

RESULTS

Malaria incidence: The incidence of malaria among volunteers is shown in Table 1. From October 1991 to April 1992 the overall incidence of malaria was 412 cases/1,000 compared with 77 cases/1,000 from April 1992 to October 1992. Malaria incidence in the 3 study locations was also variable, with more cases occurring in the central location. During the October 1991 to April 1992 period, the incidence of malaria among volunteers with permethrin-treated uniforms was significantly greater than the placebo group in the central ($\chi^2 = 14.30$, df = 1) and west ($\chi^2 = 6.06$, df = 1) locations. In the east location, volunteers who had placebo-treated uniforms had more malaria ($\chi^2 = 16.94$, df = 1) than the permethrin-treated uniform group. When the numbers of cases in both treatment groups were combined, there was no significant difference between them ($\chi^2 = 0.95$, df = 1). During the April 1992 to October 1992 period, the incidence of malaria fell, and there were no significant differences between the 2 groups when tested in each location individually or in all soldiers in the study ($\chi^2 = 0.58$, df = 1).

Field bioassays: The knockdown of *An. dirus* 1 h after a 10-min exposure to permethrin-treated shirts was 99.4% (range 94.4–100%, $n = 9$) 1 day after treatment, 39.0% (0–100%, $n = 8$) after 55 days, 66.0% (33.3–100%, $n = 8$) after 90 days, and 0% ($n = 4$) after 180 days. In these tests

Table 1. Incidence of malaria in Thai troops whose uniforms were treated with permethrin or placebo formulation, Sisaket Province, Thailand, October 1991–October 1992.

		October 1991–April 1992				April 1992–October 1992			
		No. of troops			Malaria incidence (%/6	No. of troops			Malaria incidence (%/6
Treatment	Location	Total	PF[1]	PV[2]	months)	Total	PF[1]	PV[2]	months)
Permethrin	East	46	3	1	8.7	30	0	0	0
	Central	54	32	13	83.3	34	1	4	14.7
	West	37	11	1	32.4	48	2	0	4.2
	Total	137	46	15	44.5	112	3	4	6.3
Placebo	East	95	35	6	43.2	50	1	1	4.0
	Central	106	44	12	52.8	56	1	9	17.9
	West	65	6	2	12.3	42	0	1	2.4
	Total	266	85	20	39.5	148	2	11	8.8

[1] PF = *Plasmodium falciparum* infection.
[2] PV = *Plasmodium vivax* infection.

knockdown of mosquitoes exposed to placebo-treated shirts was 0–3%.

The biting assay results (Table 2) are noteworthy as they show that both permethrin- and placebo-treated cloth deterred *An. dirus*. Less than 10% of mosquitoes exposed obtained a blood meal, whereas >50% of mosquitoes fed to repletion on bare skin. High knockdown of mosquitoes exposed to a bare thigh in the first round of tests (26.7%) was due primarily to mechanical damage following exposure.

Laboratory bioassays and chemical assays: Mean knockdown results following exposure to fabrics after hand washing is shown in Table 3. Knockdown of *An. dirus* exposed to untreated fabric was 0%, and to unwashed permethrin-treated fabric was 100%. These results indicate that there was little available permethrin remaining following 3 cold water hand washes. However, chemical analysis (Table 4) showed that 28.7–59.9% of the original permethrin remained in the fabric after 3 washes.

DISCUSSION

The wearing of permethrin-treated military clothing without repellents or other personal vector control methods did not reduce the incidence of malaria. Malaria transmission in the study area was high, as documented by our own observations and by a history of 250–400 cases/1,000/year per deployment since 1985 (C. Eamsila, unpublished data). In studies to evaluate the use of intervention measures to prevent malaria, the activities of the individuals involved influence the outcome of the control measure. For example, during a study of the effectiveness of permethrin-impregnated bednets in Kenya, random checks showed only 70% of potential users were actually sleeping under the nets (Sexton et al. 1990). During our study, the volunteers were aware of the risks of malaria and of the potential benefits from wearing the uniforms correctly to minimize mosquito bites, however it was not possible to ensure their compliance at all times.

Table 2. Biting response and knockdown (KD) of *Anopheles dirus* adults following 5 min of exposure to permethrin- and placebo-treated shirts, and to bare skin.

	Percentage *An. dirus* bloodfed and knockdown											
	1 day[1]			55 days			90 days			180 days		
Treatment	*n*[2]	Fed	KD	*n*	Fed	KD	*n*	Fed	KD	*n*	Fed	KD
Permethrin	71	2.8	97.2	168	0	73.8	111	0	79.3	36	5.6	2.8
Placebo	72	9.7	5.6	139	5.0	7.9	97	3.1	7.2	33	9.1	0
Bare skin	15	60.0	26.7	29	75.9	10.3	13	53.8	7.7	22	81.8	0

[1] Days after application of permethrin to cloth.
[2] No. of mosquitoes tested.

Table 3. Knockdown of *Anopheles dirus* following exposure to permethrin-treated fabrics after hand washing in the laboratory.

	Mean percentage knockdown[1]						
	Washes[2]						
Fabric type	0	1	2	3	4	5	6
Camouflage light green	100	85.0	44.4	0	0	2.5	2.5
Twill dark green	100	82.1	29.7	17.9	15.8	17.9	7.5
Twill light green	100	94.9	64.9	16.7	7.5	7.3	0

[1] Mean of 8 groups, each of 5 mosquitoes.
[2] No. of washes following permethrin application.

Another difficulty in interpreting the results of intervention measures is the unpredictability of malaria prevalence over time, and from location to location (Curtis 1992). In the current trial the incidence of malaria varied at each location, and could be a source of difference in the risk of exposure between the groups. Despite problems of controlling the volunteers' adherence to proper wear of treated uniforms, we feel that the study was conducted under conditions that were representative of actual, operational military activities. Troops were deployed under primitive conditions, were constantly on duty, and performed patrols under a real combat threat.

Although the objective of this study was to determine the effectiveness of permethrin-treated uniforms as protection against malaria, data on malaria transmission provided a noteworthy example of the importance of this disease to military operations. In spite of chemoprophylaxis and the use of bednets, troops suffered 412 cases/1,000 of malaria during 6 months from October 1991 to April 1992. The focal nature of malaria transmission was also demonstrated. In the first part of the study, incidence varied from 196 cases/1,000/6 months in the west location to 631 cases/1,000/6 months in the central location, even though the sites were less than 50 km apart. The relative incidence of *P. vivax* was higher in the central location (25% of malaria infections) than in the west (15%) or east (16%) locations. This trend was repeated in April to October 1992 (Table 1). Resistance of *P. vivax* to chloroquine has been reported recently in Papua New Guinea (Rieckmann et al. 1989) and Indonesia (Schwartz et al. 1991). However, a recent study suggested that chloroquine is still very effective against *P. vivax* in Thailand (Bunnag et al. 1994). The high incidence of *P. vivax* malaria in the central location during the current study may be due to the poor use of chemoprophylaxis by some volunteers.

The field bioassays showed that permethrin remained active in the shirts for up to 90 days, however it was not possible to conduct chemical analysis of these shirts. The wide range of mortality in mosquitoes exposed to permethrin-treated shirts 55 days (range 0–100%) and 90

Table 4. Chemical analysis of permethrin-treated cloth following hand washing.

	Permethrin content (mg/g ± SE)[1] following washing		
	Washes		
Fabric type	0	3	6
Camouflage light green	12.32 ± 4.68 (100)[2]	3.73 ± 0.49 (30.3)	3.18 ± 0.18 (25.8)
Twill dark green	12.02 ± 1.42 (100)	7.2 ± 0.56 (59.9)	7.85 ± 0.48 (65.3)
Twill light green	13.03 ± 0.40 (100)	3.74 ± 0.26 (28.7)	3.33 ± 0.12 (25.6)
Unwashed treated control (camouflage)	—	7.28 ± 0.56	7.94 ± 0.17

[1] Units of mg/g are approximately mean × 0.021 mg/cm² for the camouflage cloth, and mean × 0.035 mg/cm² for the twill fabrics.
[2] Number in parentheses shows the percentage of active ingredient remaining in the fabric compared to the original amount applied.

days (33.3–100%) after treatment suggests that washing and wear of uniforms under field conditions varies greatly. In the laboratory, permethrin-treated shirt fabric had minimal activity against *An. dirus* following 3 hand washes. Chemical analysis of subsamples of this material showed that 28.7–59.9% of the original active ingredient remained after 3 washes (Table 4), but the knockdown of *An. dirus* was less than 20% (Table 3). These results suggest that investigation of optimal retreatment regimes under field conditions is warranted.

Gupta et al. (1989) showed that permethrin diminished rapidly from treated fabric following accelerated weathering in the laboratory, but permethrin-treated fabrics provided >93% protection against *Anopheles stephensi* Liston, with 92% provided by untreated fabric. In a similar study, Gupta et al. (1990) reported permethrin provided good protection from biting mosquitoes, and untreated fabric also provided protection from biting, although this was more variable. The type of fabric was also important, with untreated 100% cotton providing an average of 95.5% protection against *An. stephensi,* compared with 91.8% provided by untreated 50% nylon–50% cotton fabric (Gupta et al. 1990). The results of the current trial show that *An. dirus* responds in a similar way to clothing fabric. In biting bioassays <10% of *An. dirus* obtained blood through the placebo and treated shirts. Knockdown on treated cloth of probing mosquitoes was greater than that following exposure to the same shirts by tarsal contact. The knockdown of probing mosquitoes was 73.8%, 79.3%, and 2.8% at 55, 90, and 180 days posttreatment (Table 2) compared to knockdown of 39.0%, 66.0%, and 0% for mosquitoes exposed by tarsal contact.

The use of permethrin sprayed onto military uniforms did not reduce the incidence of malaria in troops deployed to an area endemic for drug-resistant *P. falciparum* malaria.

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