Efficacy of a New Self-Supporting Low-Profile Bednet for Personal Protection Against *Anopheles farauti* (Diptera: Culicidae) in a Village in Papua New Guinea

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ABSTRACT A new United States (U.S.) self-supporting low-profile bednet was designed by Walter Reed Army Institute of Research in collaboration with Breakthrough Technologies. The bednet incorporated permethrin-impregnated screening into a frame that erected automatically when removed from its bag. The new U.S. bednet was compared with the current Australian Defense Force (ADF) mosquito bednet at Buka Island, North Solomons Province, Papua New Guinea, in March 1999. At the time of the test, *Anopheles farauti* Laveran was the most abundant biting mosquito. Both bednet types provided >97.8% protection compared with an unprotected collector. The untreated U.S. Army prototype bednet provided better protection than the untreated ADF bednet against mosquitoes entering the bednet during the night.

KEY WORDS *Anopheles farauti*, Papua New Guinea, bednet, malaria, personal protection, permethrin

BEDNETS IMPREGNATED WITH permethrin or other pyrethroids have become a common intervention measure for reducing exposure to vectors of malaria (Rozendaal 1989, Curtis 1991, Kere et al. 1996). This personal protection measure is also used by nonimmune military forces deploying to malarious areas. While the most important aspect in the use of bednets by soldiers is to create a mechanical barrier against biting mosquitoes, other issues of a tactical nature must be considered. The bednets must have a low profile, be easy to enter and exit from quickly, and be compact and lightweight. In many cases, patrolling soldiers need to be able to erect bednets nightly, often in the dark, so it is important that bednets can be simple to set up. The bednets currently used by the United States (U.S.) and Australian military have four tie off points that are designed to hold the bednet above a cot fitted with four wooden poles. The use of poles is inconvenient, as they add ≈1.5 kg to the weight that a soldier has to carry and are cumbersome in the field. Furthermore, current military bednets are difficult to use without a cot and require at least 5–10 min to set up properly. Bednets are often erected incorrectly, compromising their effectiveness. In an attempt to eliminate the need for tie off points, the U.S. Army has developed a self-supporting prototype bednet that can be deployed quickly, is lightweight (<1 kg), and has a low profile.

In an earlier study in the Amazon basin in Peru, the new prototype bednet (permethrin treated and untreated) and current issue U.S. military permethrin-treated bednets provided significantly better protection against mosquito bites than untreated current issue military bednets (Gupta et al., unpublished data).

Over the last decade, in support of United Nations peace-keeping initiatives, Australian soldiers have frequently been deployed to areas in which vector-borne diseases such as malaria and arboviruses are a problem, making mosquito bednet use essential. Recently, Australian Defense Force (ADF) soldiers were deployed to North Solomons Province (Buka and Bougainville Islands), Papua New Guinea (PNG), as part of a multinational peace-monitoring group. PNG is highly malarious, and the use of all forms of personal protection and antimalarial drugs is mandatory (Cattani et al. 1986, Rieckmann et al. 1993). During this deployment, the opportunity was taken to compare the effectiveness of a new prototype U.S. military bednet and current issue ADF bednets in protecting sleeping people from nuisance mosquitoes and vectors of malaria.

The views of the authors do not purport to reflect the position of the Australian Defense Force (ADF) or Department of Defense (Australia). Mention of a commercial product does not constitute an endorsement of the product by the ADF. The volunteers gave informed consent to participate in the study.

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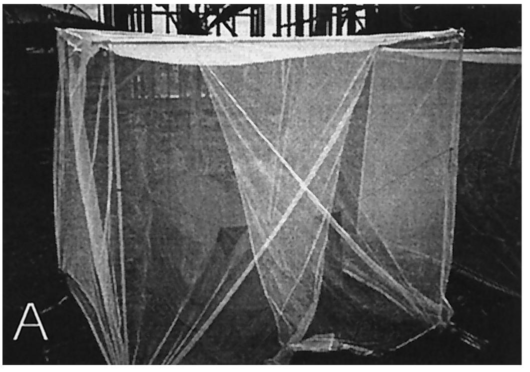

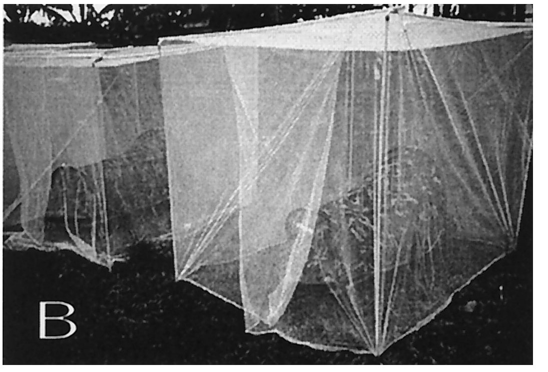

Fig. 1. ADF bednet (A) and U.S. Army prototype bednets (B), erected inside large family-sized bednets in the field at Buka, PNG.

Materials and Methods

Field Study

Study Site. The study was conducted in the town of Suharno on Buka Island (5° 26′ S, 154° 40′ E), North Solomons Province, PNG, in March 1999.

Test Bednets. Two types of mosquito bednets were used:

1. ADF mosquito bednet made from nylon (mesh size, 43 holes/cm^2), and when erected are 0.55 m high, 0.75 m wide, and 2.1 m long. This bednet does not have a floor, so access is achieved by raising a side of the bednet and moving in or out.

2. A U.S. prototype self-erecting, low-profile mosquito bednet and shelter manufactured by Breakthrough Technologies (Winston-Salem, NC). The bednet has a taffeta floor and nylon polyester net (mesh size, 410 holes/cm^2), and is 0.68 m high, up to 0.8 m wide, and 2.0 m long. Access to the bednet is by a zippered side opening.

An ADF bednet was treated by dipping in an emulsion containing 0.6% permethrin from Peregin, a 500 g/liter formulation containing 25:75 *cis:trans* of permethrin (Frances et al. 1992). A second ADF bednet was untreated. A single prototype U.S. Army bednet was manufactured and treated to have a final concentration of 0.12 mg/cm^2 of permethrin in the fabric. A second U.S. prototype bednet was untreated.

Test Procedure. Four large outer mosquito bednets, 2.0 × 2.0 × 1.8 m (Siam Dutch Co., Bangkok, Thailand), were erected in the study area in a straight line, ≈60 cm apart. Under each of these bednets (called outer bednets), a standard ADF folding cot was set up, and over these the four test mosquito bednets were erected (Fig. 1), similar to the method of Charlwood et al. 1986. During the test periods, the outer bednets were raised ≈15 cm above the ground to allow mosquitoes to enter.

On each of four test nights, an individual entered each test bednet at 1900 hours, and remained in the bednet for up to 10 h, with a 30-min break taken at 2200 hours. After this break, all individuals returned to the bednets at 2230 hours and remained in them until 0600 hours the following morning.

Mosquitoes were collected hourly from inside each outer bednet over the period 1900–0600 hours. These collections commenced 45 min after the individuals entered the test bednets, so that 10 collections were made from each outer bednet on each of the four nights. The collections were made using an aspirator and flashlight by two entomologists collecting alternately from two of the four bednets for 15 min each hour. At the conclusion of each night of testing, the inside of each test bednet was examined and any mosquitoes were collected. A sample of *Anopheles* sp. was identified using polymerase chain reaction (PCR)-restriction fragment-length polymorphism (RFLP) (PCR-RFLP) analysis (Beebe and Saul 1995). To obtain information on the human biting rate of mosquitoes during the study, a single collector conducted human biting catches for 30 min each hour, commencing at 1900 hours and concluding at 0530 hours, the following day. To obtain information on malaria infection rates, a sample of *Anopheles farauti* Laveran was tested for the presence of circumsporozoite proteins using a specific enzyme-linked immunosorbent assay (Wirtz et al. 1985).

Effects of Permethrin. All mosquitoes were kept in their original collection cup and returned to the field laboratory at the conclusion of each night of testing. Mosquitoes collected from the outer bednets between 0200 and 0600 hours had a pad of moist cotton wool placed onto the cup, and knockdown was recorded 6–9 h after collection. However, because of the absence of suitable storage facilities, it was not possible to hold mosquitoes in the original cups to record mortality after 24 h.

Laboratory Studies. The ability of 6- to 7-d-old laboratory-reared *An. farauti* to obtain a blood meal through permethrin-treated and untreated bednet material was tested using the method of Frances and Sweeney 1996. Mosquitoes from a colony established from specimens collected in Rabaul, PNG, in 1972, were held in groups of about 100 in one-gallon wax paper cups and provided with a sugar pad and moist cotton wool for 3–4 d before the tests. Batches of 10 adult mosquitoes were used, and placed into wax paper cups (7 cm diameter) covered with un-

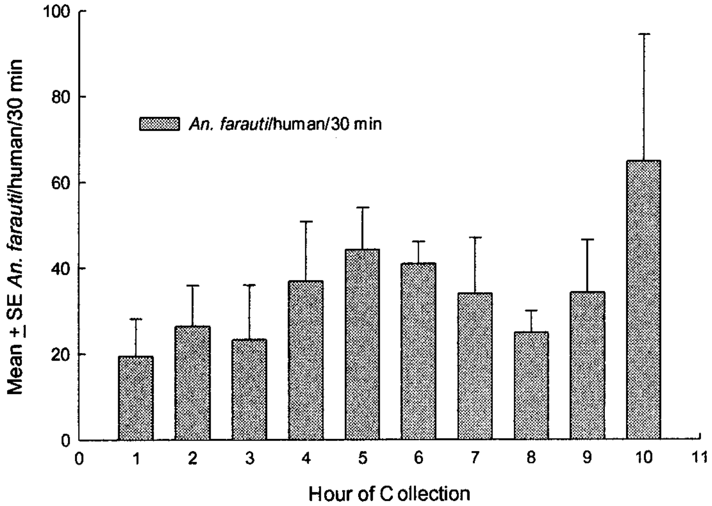

Fig. 2. Mean (± SE) number of *An. farauti* collected hourly from an individual collector at Buka, PNG, 6–10 March 1999. Hour 1–4 is 1900–2200 hours; hour 5–10 is 2400–0500 hours.

treated ADF bednet material. Then, test mosquitoes were transferred into cups covered with permethrin-treated and untreated material cut from the ADF and U.S. prototype bednets. Permethrin-treated U.S. bednet material used was obtained from factory-treated material, and permethrin-treated ADF bednet material from the top of an unused bednet treated at the same time as the test bednet used in the field study. The cups were then pressed to the naked stomach of a Caucasian male volunteer, and the mosquitoes were allowed to feed for 10 min. Mosquitoes were then aspirated from the test cups and returned to holding cups covered with a moist cotton wool pad. The number of mosquitoes bloodfed was recorded. For all mosquitoes, the knockdown was scored at 1 h and mortality after 24 h. A minimum of three batches of mosquitoes was exposed to each bednet type. A sample of treated swatches was chemically analyzed for permethrin content.

Results

Field Study. Overall, 1,207 human biting mosquitoes were collected during the tests, and 1,094 (90.6%) of these were identified as *An. farauti* using PCR-RFLP analysis. The relative abundance of mosquitoes was fairly uniform throughout the duration of the collections (Fig. 2). Using a one-way analysis of variance, there were no statistical differences between the mean number of mosquitoes biting an unprotected collector each hour ($F = 1.41$; df = 9,30; $P = 0.24$).

A total of 1,253 mosquitoes were collected from the inside of the outer bednets, of which 576 (46.0%) were *An. farauti.* Overall, a mean of 9.73 ± 0.70 mosquitoes were collected per bednet/h and 4.50 ± 0.41 *An. farauti*/h. The total number of bloodfed mosquitoes from the outer bednets was 63 of the 576 (10.9%) *An. farauti* and 11 of the 677 (1.6%) *Culex* sp collected. There were no differences in the total number or number of bloodfed mosquitoes collected from the four different bednet types (Table 1). A total of 55 mosquitoes were collected from inside the untreated ADF bednet, comprising 52 *An. farauti* and 3 *Culex* sp., of which 41 (78.9%) were bloodfed. Two *An. farauti* were found inside the treated ADF bednets, with one bloodfed. In the U.S. bednets, a single *Culex* sp. adult was collected unfed from the untreated bednet, while no mosquitoes were collected inside the permethrin-treated U.S. bednet.

A total of 787 *An. farauti* were tested for the presence of circumsporozoite proteins using a specific enzyme-linked immunosorbent assay. Twelve (1.5%) mosquitoes were found with *Plasmodium falciparum* (Welch) sporozoite proteins, and five (0.6%) with the 210 variant of *Plasmodium vivax* (Grassi and Feletti), indicating a relatively high rate of malaria transmission in the study area.

The mortality of mosquitoes ($n = 391$) collected from the outer bednets and held for 6–9 h is shown in Table 2. The mortality of mosquitoes collected in untreated and treated ADF bednets ($\chi^2 = 0.001$; df = 1; $P = 0.97$) and U.S. prototype bednets ($\chi^2 = 0.19$; df = 1; $P = 0.66$) was not significantly different, indicating

Table 1. Mean ± SE number of *An. farauti* collected in outer nets covering each bednet type at Buka, PNG, 6–10 March 1999 ($n = 576$)

Bednet type	*An. farauti* collected per hour	
	Bloodfed	Total
Untreated ADF	0.50 ± 0.17a	5.3 ± 1.1a
Treated ADF	0.16 ± 0.07a	3.5 ± 0.5a
Untreated US prototype	0.69 ± 0.27a	4.6 ± 0.7a
Treated US prototype	0.56 ± 0.43a	4.5 ± 0.9a

Within column means followed by the same letter are not significantly different using Kruskall Wallis Ranks one-way analysis of variance and Student Newman Kuels method, $P < 0.05$.

Table 2. Mortality of mosquitoes collected from outer nets 6–9 h after collection

Time after collection (hours)	Untreated ADF		Treated ADF		Untreated US prototype		Treated US prototype	
	Living	Dead	Living	Dead	Living	Dead	Living	Dead
6	14	1	20	0	17	3	20	0
7	13	0	11	0	20	2	12	5
8	46	4	48	5	37	6	29	6
9	15	1	12	0	27	1	15	1
Totals (% mortality)	88	6 (6.8)	91	5 (5.5)	101	12 (11.9)	76	12 (15.8)

that the permethrin treatment did not have a short-term effect on these mosquitoes.

Laboratory Studies. The concentration of permethrin in two samples of treated U.S. prototype bednet material was 0.797 and 0.574 mg/cm^2. A sample of material from the top of the treated Australian bednet was 0.138 mg/cm^2. The ability of laboratory-reared *An. farauti* to feed through bednet material is shown in Table 3. Despite the presence of permethrin in the fabric of both types of bednets, 31.7% of *An. farauti* obtained a blood meal through the U.S. prototype bednet and 93.3% through the ADF bednet fabric.

Discussion

The results of this study have shown that the U.S. Army prototype bednet and ADF bednet provided similar protection against *An. farauti*, an important malaria vector in PNG. When compared with an unprotected collector, both bednets provided >97.8% protection. Studies conducted in Tanzania have shown that biting rates of *Anopheles gambiae* Giles are reduced by 95% with the use of intact insecticide-treated bednets (Lines et al. 1987, Gokool et al. 1992). However, despite this increase in protection, a relatively high number of mosquitoes collected in the large outer bednets were blood engorged (Table 1), indicating that these mosquitoes were able to feed through the net on the sleeping volunteer. Previous studies have shown that *Anopheles* mosquitoes can bite through permethrin-treated nets (Hossain and Curtis 1989, Frances and Sweeney 1996).

To determine the source of bloodmeals of mosquitoes collected in hut trials of insecticide-treated bednets in Tanzania, a PCR method was used to match the DNA profile of the blood in the mosquitoes with the profile of individual sleepers. In one study, the majority of bloodfed mosquitoes had fed on people other than the sleepers (Gokool et al. 1992). However, in a subsequent trial, 89.7% of mosquitoes had fed on sleeping individuals (Gokool et al. 1993). It was not possible to identify the blood source of engorged mosquitoes collected in the outer bednets in the current study. However, we assume that the volunteers had moved into contact with the net material, allowing mosquitoes to feed, showing the importance of the sleeper minimizing contact with the bednet during the night. There was no difference in the protection provided by all bednets against mosquitoes outside the bednets, but the untreated ADF bednets allowed a total of 55 mosquitoes to enter the bednet during the tests, while only a single *Culex* mosquito was collected inside a U.S. bednet. This increase in protection provided against mosquitoes entering the U.S. bednets can be attributed to their structure. The U.S. bednets have a floor that fully encloses the sleeper, whereas the ADF bednet does not have a floor, and after movement of the sleeper may be disrupted and allow opening for the random entry of mosquitoes. Although the mosquitoes collected from the outer bednets were not affected by permethrin, there were fewer mosquitoes collected inside bednets treated with permethrin at the end of each night of testing. There were only two mosquitoes collected inside the permethrin-treated ADF bednet during the tests, and no mosquitoes were collected inside permethrin-treated U.S. prototype bednets, supporting previous studies showing the benefit of treating bednets with permethrin (Rozendaal 1989).

The night biting activity of *An. farauti* has been found to change in several areas as a result of indoor residual dichlorodiphenyltrichloroethane (DDT) spraying. Studies conducted in Guadalcanal, Solomon Islands, showed that *An. farauti* developed an early night biting pattern to avoid DDT-treated surfaces (Taylor 1975). On Buka Island, DDT spraying was routinely carried out for over 20 yr up until the early 1980s. However, the results of the current study indicate no change in the night biting pattern, with *An. farauti* biting uniformly throughout the night (Fig. 2).

In conclusion, this study has shown both bednet types provided >97.8% protection compared with an unprotected collector, against *An. farauti,* and there-

Table 3. Biting response, knockdown, and mortality of *An. farauti* following 10 min of exposure to bed net material untreated and treated with permethrin

Bednet type	Number of mosquitoes	Fed (%)	% knockdown 1 h after exposure	% mortality 24 h after exposure
Untreated ADF	50	70.0	0	0
Treated ADF	30	93.3	56.7	6.7
Untreated US prototype	90	48.4	0	0
Treated US prototype	60	31.7	100	78.3

fore there is considerable value in using a bednet to reduce the risk of contracting malaria. The untreated U.S. Army prototype provided better protection than the untreated ADF bednet against mosquitoes entering the bednet during the night.

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