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Comparison of permethrin treatments for bednets in The Gambia

R.J. Pleass, J.R.M. Armstrong, C.F. Curtis
London School of Hygiene and Tropical Medicine, London, UK.

M. Jawara and S.W. Lindsay
Medical Research Council Laboratories, Fajara, The Gambia

Abstract

An experimental hut trial of bednets was undertaken in The Gambia to compare six different permethrin treatments, including a wash-resistant formulation, and to test the effect of thorough hand washing of the nets. The wash-resistant formulation on nylon and cotton nets deterred *Anopheles gambiae* Giles (*sensu lato*) from house entry. All treated bednets killed a significantly higher number of *A. gambiae* (*sensu lato*) than untreated nets. The ability to kill mosquitoes was not diminished with the wash-resistant and Wellcome formulations after washing unlike the normal ICI formulation, this result being consistent with the permethrin residues found by gas liquid chromatographic (GLC) analyses to persist on the nets after washing. The mortality on treated cotton nets was significantly less than on treated nylon nets. Of the unwashed nets only the cotton net was significantly repellent. Both washed and unwashed treated nets significantly reduced the proportion of human blood-fed mosquitoes that survived and were potentially able to transmit malaria. Although the wash-resistant formulation might prove more efficient at killing mosquitoes with time, as compared to the other treatments, in The Gambia, where women wash their nets frequently, we suggest that the cheapest and most readily available formulation is used on nylon nets where possible.

Introduction

Impregnating bednets with residual insecticides such as permethrin is effective against malaria mosquitoes (Curtis *et al.*, 1989; Rozendaal, 1989; WHO, 1989) as well as other biting arthropods (Charlwood & Dagaro, 1988; Lindsay *et al.*, 1989).

Permethrin is in many ways an ideal insecticide. Its ability to kill mosquitoes effectively, its low toxicity to man and its bio-degradability, but good persistence on bednets, make it the insecticide of choice (Curtis *et al.*, 1989; Rozendaal, 1989; WHO, 1989).

Correspondence: Mr R.J. Pleass, The Wellcome Research Centre for Parasitic Infections, Imperial College of Science Technology & Medicine, The West Wing, Prince Consort Road, London, SW7 2BB, UK.

Finding the cheapest and most effective formulation of permethrin for treating cotton or nylon netting that can withstand the rigours of washing is important if bednets are to be incorporated into malaria control programmes. A formulation of permethrin incorporating polystyrene into the ICI emulsifiable concentrate was shown by repeated bioassays to withstand washing better than the standard formulation (Miller, 1990).

A major trial conducted in The Gambia at the time of this study involving 73 villages and some 22,000 people, reduced mortality in children 1 to 4 years old by 63% (Alonso *et al.*, 1991). This trial was so successful that the Gambian government plans to introduce a national control programme using treated nets and the World Health Organisation is planning further trials in Africa involving 150,000 people (WHO, 1991).

The aims of the present experimental hut trial were twofold. First, to determine whether before and after washing, the wash-resistant permethrin was more effective against mosquitoes than nets treated with normal emulsifiable concentrates from two major insecticide manufacturers. Second, to compare the wash-resistant formulation on nets made of cotton and nylon.

Materials and methods

Study area

The investigation took place at Wali Kunda, a field station run by the British Medical Research Council, in the MacCarthy Island district of The Gambia. The Field Station is on the south bank of the River Gambia, approximately 300 km from the coast. Mosquitoes breed in the rice fields to the south of Wali Kunda and are abundant during the rainy season (Lindsay *et al.*, 1991a). The bednets were tested in verandah trap experimental huts in an area of open woodland with typical Sudan savanna vegetation. The trial lasted six weeks from the 15th July to the 25th August 1990 with a one night break on Saturday each week. This period coincided with the start of the rains.

Experimental huts

Six experimental huts were built in a line 12 ms apart orientated along an East-West axis. The design and dimensions were described by (Miller, 1990), based on traditional African houses with verandahs extending from each of the four walls. The eaves were open on all sides of the hut, but those on the east and west sides opened into verandahs screened with mosquito netting. Exit traps were placed over open windows on the two walls of the screened verandahs. Each hut was raised from the ground on concrete pillars that had water channels around them. These moats prevented the entry of ants and subsequent scavenging of knocked down mosquitoes. On each rest day a 1:1 borax : glucose solution was placed in the huts to kill any ants evading the ant traps. The ant baits were removed on the days of the trial.

Bednets

Pairs of bednets were given the following target doses:

1. 500 mg normal ICI permethrin/m^2 of nylon net (From 25% Emulsifiable concentrate (E.C.), 'Imperator').
2. 500 mg normal Wellcome permethrin/m^2 of nylon net (from 10% E.C., 'Peripel').
3. 500 mg wash-resistant ICI permethrin/m^2 of nylon net (from 25% E.C., plus 5% polystyrene 100,000 m.w., BDH).
4. 500 mg wash-resistant ICI permethrin/m^2 of cotton net.
5. 1000 mg wash-resistant ICI permethrin/m^2 of nylon net.

A further pair of untreated nylon bednets served as controls. One of each pair of bednets remained unwashed whilst the other was hand washed.

The cotton and nylon bednets had a 1 mm mesh and weighed 42 and 31 g/m^2 respectively, when dry. Both types of net were 10.2 m^2 in area and were purchased from the Swiss Net Company (Nottingham, UK).

The volume of water each net absorbed was determined by weighing the net dry, saturating it in distilled water for 15 minutes, wringing out the excess water and reweighing the net. The nylon nets absorbed a mean of 415 ml of water and the cotton net 794 ml. The amount of insecticide required for each treatment was calculated using the following formula:

$$\text{Amount of emulsifiable concentrate (E.C.) mls} = \frac{\text{target deposit density (g/m}^2\text{)} \times \text{area of fabric (m}^2\text{)} \times 100}{\text{\% of active ingredient in the E.C.}} \quad (1)$$

The required amount of E.C. was mixed with 830 ml of water for each nylon net or 1588 ml for each cotton net. Each net was left to soak for 5 min in the insecticide emulsion, wrung out by hand and left to dry indoors horizontally on polythene bags.

Six holes, each 10 x 10 cm, were cut from each net, two at each side and one at each end, to simulate badly torn bednets. These samples were used for GLC analysis of the permethrin deposit densities (Lindsay *et al.*, 1991b); this analysis was repeated on pieces cut from the nets after the trial.

At the start of the trial, one of each of the six pairs of nets was hand washed three times on separate days by different Gambian women. During a single wash the net was rubbed with local cow fat soap and rinsed twice that took approximately 7 minutes. The nets were handled as little as possible and were dried indoors to avoid possible degradation of insecticide by ultraviolet.

Six healthy Gambian men aged between 25 and 55, were employed as sleepers. They went under the nets at 22.00 h and remained there until 07.00 h. The men were given chloroquine prophylaxis weekly.

The experimental design was based on a series of Latin squares (Cochran & Cox, 1957), to allow for possible variation between huts, sleepers, nights of study and between treatments. Briefly, sleepers were allocated to a different hut for each week of the study, and the five treated nets and the untreated net, either washed or unwashed, were allocated to different huts on each night of the week. The twelve nets were each tested 18 times. The nets were coded at the start of the trial to enable the trial to be carried out 'double blind', although the cotton nets were seen to differ from nylon nets.

Collection and identification of mosquitoes

Each hut was searched with torches and aspirators for 60 min each morning; 20 min for each room and 20 min for each of the two enclosed verandahs with the exit traps.

The principal vectors of malaria in the study area were freshwater members of the *A. gambiae* (Giles) complex; consisting mainly of *A. gambiae* (*sensu stricto*) with few *A. arabiensis* (Patton) (Lindsay *et al.*, 1991a).

Mosquitoes were scored as blood-fed or unfed, and dead or alive. Blood-meals were identified as human or non-human using a modification of the ELISA technique described by Burkot *et al.* (1981). On the last day of the trial the collecting efficiency of the mosquito collectors was assessed. After carrying out the usual visual search of 60 minutes per hut, each hut was sprayed with an insecticide aerosol containing d-allethrin and d-phenothrin. The hut was then searched for mosquitoes. The collecting efficiency for two catchers was 88%.

Statistical analysis

Counts of mosquitoes were log transformed (ln [n+1]) and proportions angularly transformed ($\sin^{-1}\sqrt{P}$) to normalize the distribution and stabilize the variance. Analysis of variance (ANOVA) was carried out using the statistical package GLIM (Payne, 1986) to partition variation into components for nights, huts, sleepers and treatments. Since only six of the twelve treatments were tested on any one night, the results given are adjusted for the night of the trial. Estimated numbers or proportions of mosquitoes found in each hut each morning were adjusted relative to those found in hut one (sleeper one and net one) at the start of the study. Where the ANOVA showed significant variation between treatments, standard errors of the difference between any two means, and t-tests, were used to compare pairs of treatments. Where two treatments differed by more than two standard errors, the difference was considered significant ($P < 0.05$). The standard error of the difference between any two means (SED) may be derived using the formula shown below:

$$SED = \sqrt{2s^2/n} \qquad (2)$$

where the sample size on which each the two means are based is denoted by n.

The following response variables were measured:

1. Total number of *A. gambiae* (*sensu lato*) entering the huts overnight, a measure of the effect of the net treatments in deterring mosquitoes from entry, was estimated by doubling the verandah trap catches (since mosquitoes could equally exit via the two open eaves or into the verandah traps) and adding this to the number found in the window traps and inside the huts.
2. Total number of *A. gambiae* (*sensu lato*) found dead or 'knocked down'. As with 1), the verandah trap collection was multiplied by two. The total number of mosquitoes killed is an indication of the likely effectiveness of the widespread use of a given net treatment for community protection.
3. The total number of *A. gambiae* (*sensu lato*) estimated to have exited the huts via the window traps and verandahs, as a percentage of the total number entering. This is a measure of the excito-repellency of the nets.
4. The total number of *A. gambiae* (*sensu lato*) found to have fed on humans. The reduction in the number of blood-feeds on humans is a measure of the personal protection that a net gives against the risk of malaria infection.
5. The total number of *A. gambiae* (*sensu lato*) that were found alive and blood-fed on man, which is important for assessing the effect of the insecticide treatment on the potential for transmission of malaria from a net user.

Results

Gas liquid chromatography analysis

Results are shown in table 1. The normal Wellcome formulation and the wash resistant formulation left more permethrin on the net after washing than did the normal ICI formulation. However, the Wellcome and wash-resistant formulations still lost about 85% of their deposit on washing of nylon nets. The loss was less from the cotton nets.

The target deposit density of 1000 mg wash-resistant ICI permethrin/m^2 was not attained and the actual deposit approximated that of the nets treated with 500 mg/m^2.

Table 1. Results of gas liquid chromatography analyses. Mean weights of permethrin in mg/m^2 with 95% confidence limits in brackets

Treatments	Target dose	Start of trial		End of trial	
		Net A Unwashed	Net B Unwashed	Net A Unwashed	Net B Washed
Untreated nylon		0	0	0	0
Normal ICI nylon	0.5 g/m^2	323 (275-371)	218 (129-307)	368 (287-449)	3.6 (2.3-4.9)
Normal Wellcome nylon	0.5 g/m^2	277 (219-335)	262 (215-309)	220 (191-249)	44 (27-61)
Wash-resistant ICI nylon	0.5 g/m^2	356 (265-447)	298 (132-464)	382 (300-464)	41 (26-56)
Wash-resistant ICI cotton	0.5 g/m^2	502 (444-560)	395 (371-419)	365 (337-393)	116 (89-143)
Wash-resistant ICI nylon	1 g/m^2	455 (400-510)	353 (272-434)	515 (405-625)	148 (113-183)

n.b. The nets designated B had least insecticide on them at the start of the trial and these were therefore chosen to be the washed nets in The Gambia.

Table 2. Results of analysis of variance (ANOVA) for *A. gambiae* (*sensu lato*)

Response analysed	Difference between treatments
	F*
Total number entering hut	3.2
Total number dead	26.3
Proportion dead	35.5
Proportion exiting	9.9
Total number human bloodfed	6.2
Total number live and human bloodfed	20.3

*F-tests are on 11 and 159 degrees of freedom; $p < 0.001$ for each response variable analysed.

ANOVA

Results between net treatments from the analyses of variance are summarised in table 2. F values were significant for all response variables analysed, and t-test comparisons between individual treatments on the basis of the standard error of the difference between means are therefore justifiable. There were significant differences between huts and nights ($p < 0.05$) for all variables analysed.

House entry

In figure 1 and all subsequent histograms, the means and approximate standard error of the difference between any two means (SED) are shown.

Of the unwashed nets, only those with wash-resistant permethrin on nylon or cotton showed evidence of significant deterrency against house entry by *A. gambiae* (*sensu lato*) (t-tests: $P < 0.02$). There was no evidence of significant deterrency with any of the washed nets. The washed nylon net treated with the wash-resistant formulation was significantly less deterrent than its unwashed counterpart (t=2.19, d.f=34, $P < 0.05$).

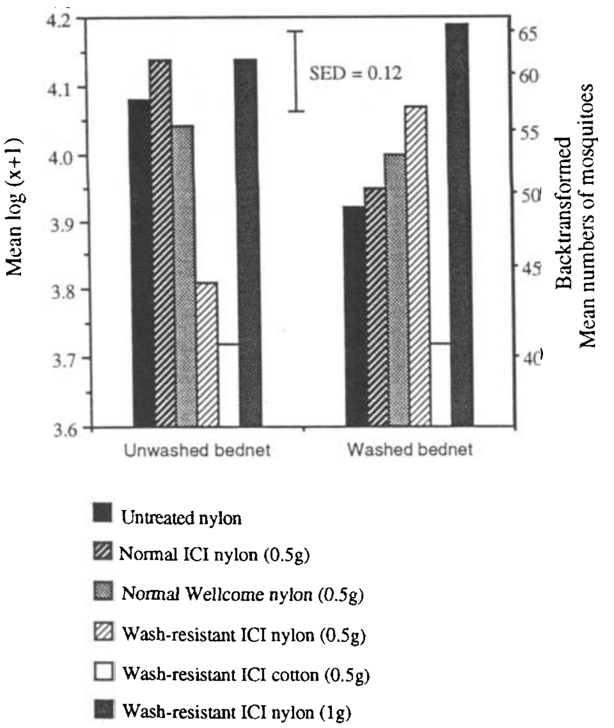

Fig. 1. Mean numbers of *A. gambiae* (*sensu lato*) entering huts each night. (The legends for all subsequent graphs are identical to those used in Figure 1.)

Mortality

As shown in figure 2(a) all the treated nets, whether washed or unwashed, killed a significantly higher number of mosquitoes than did the untreated nets (t-tests: $P < 0.05$). However the unwashed cotton net treated with wash-resistant permethrin killed significantly fewer *A. gambiae* (*sensu lato*) than the normal ICI or Wellcome formulations on nylon (t-tests: $P < 0.05$). The only treatment whose killing power differed significantly between washed and unwashed nets was the normal ICI on nylon (t=3.2, d.f=34, $P < 0.005$). There was no significant difference in killing ability between any of the formulations on the washed nylon nets (t-tests: $P > 0.05$).

The numbers found killed are expected to be affected

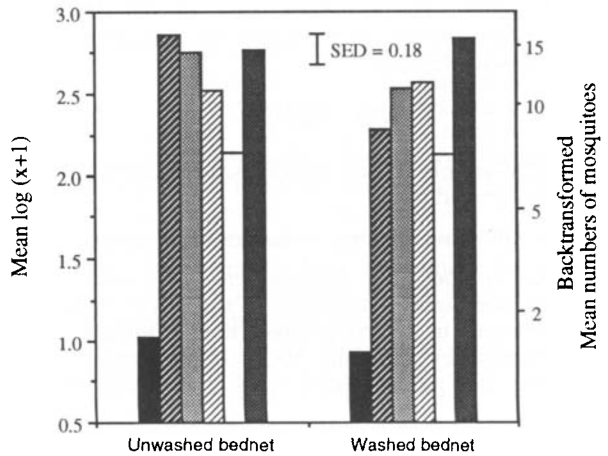

Fig. 2a. Mean numbers of *A. gambiae* (*sensu lato*) found dead per hut each night.

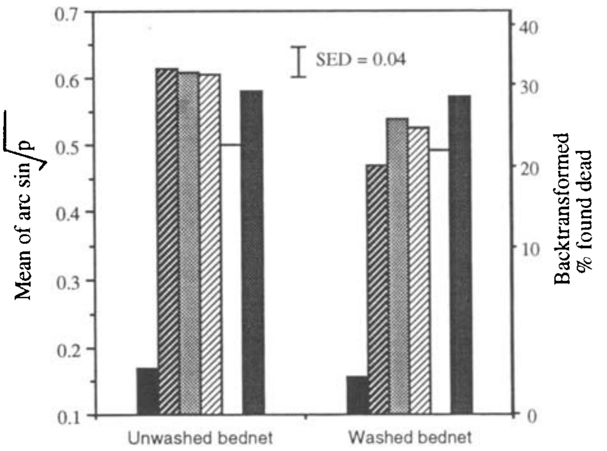

Fig. 2b. Proportion of *A. gambiae* (*sensu lato*) found dead per hut each night.

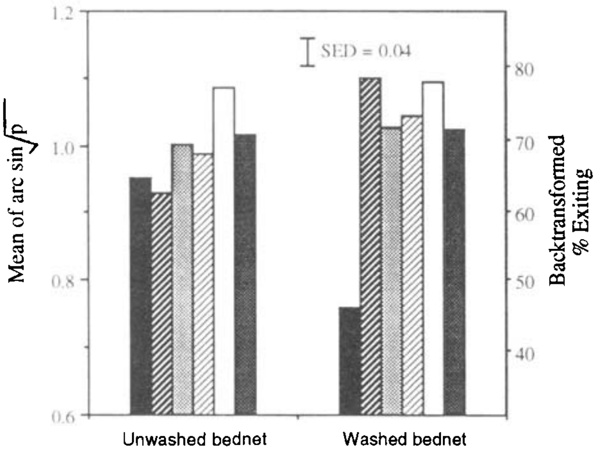

Fig. 3. Proportion of *A. gambiae* (*sensu lato*) exiting.

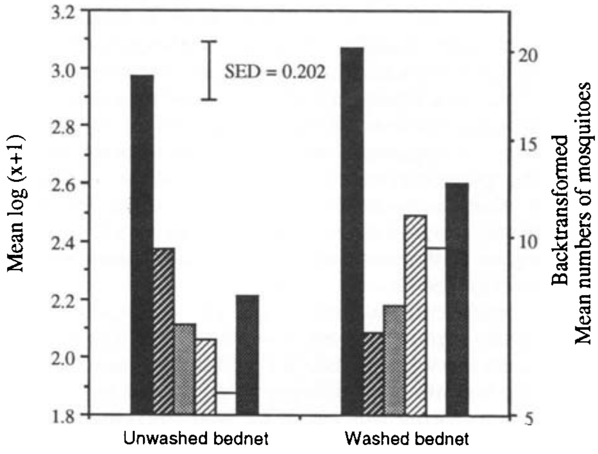

Fig. 4. Mean numbers of bloodfed *A. gambiae* (*sensu lato*) in huts each night.

by differences in numbers entering the huts. However when the proportions dead of those which entered the hut were calculated (fig. 2b), and comparisons made between the nets, all the above statements about differences and similarities remained true.

Exiting

As shown in figure 3, with the washed untreated net a significantly smaller proportion left the hut than with the unwashed untreated net (t=5, d.f=34, P <0.001). There was no significant difference in the proportions of mosquitoes exiting the huts with unwashed nets treated with any of the formulations as compared with the unwashed untreated net, with the exception of the wash-resistant formulation on cotton, which caused significantly more exiting (t-3.35, d.f=34, P <0.002).

All the washed treated nets caused significantly more exiting than the washed untreated net (t-tests: P <0.001). There was no significant difference between any of the pairs of washed and unwashed nets with the same treatments in proportion of mosquitoes exiting, except the ICI formulation on nylon which, after washing, caused significantly more mosquitoes to exit than it did before washing (t=3.1, d.f=34, P <0.005).

Blood feeding

Figure 4 shows that all unwashed and washed treated nets significantly reduced the numbers of *A. gambiae* (*sensu lato*) found bloodfed (t-tests: P <0.025) compared to the untreated net. Of the unwashed nets, the wash-resistant treatment on cotton reduced blood-feeding more than the normal ICI treatment on nylon (t=3.56, d.f=34, P <0.001). The washed cotton net was no better than the other treatments at preventing blood-feeding, and each washed net performed as well as the corresponding unwashed nets, except the wash-resistant treatments on nylon and cotton. These washed nets allowed significantly more blood-feeding than the corresponding unwashed net (t-tests: P <0.05).

In the present study, 87.2% of blood-meals by *A. gambiae* (*sensu lato*) were of human origin.

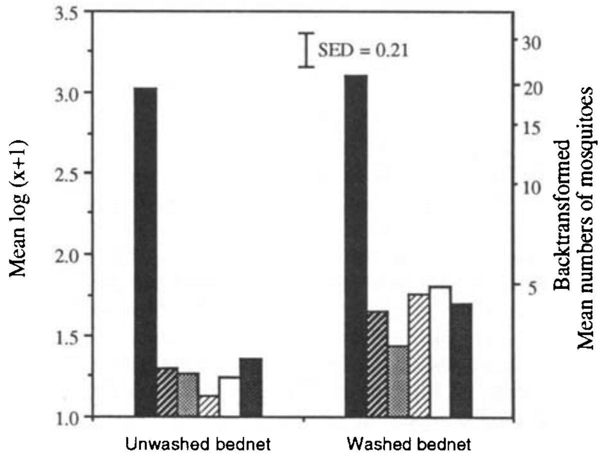

Fig. 5. Mean numbers of live human-fed *A. gambiae* (*sensu lato*) found per hut each night.

Figure 5 shows that all unwashed and washed treated nets significantly reduced the numbers of *A. gambiae* (*sensu lato*) fed on human blood and found alive (t-tests: P <0.001). Of the washed nets, the wash-resistant treatments on nylon and cotton performed less well than their unwashed counterparts and this difference was significant (t-tests: P <0.001).

Discussion

The cotton nets caused most deterrency both before and after washing probably because they absorbed more of the formulation than other nets. Recent evidence suggests that deterrency results from volatile components in the emulsifiable concentrate, rather than the insecticide itself (Lindsay *et al.*, 1991c). The polystyrene in the wash-resistant formulation is also suspended in an emulsifiable concentrate that may explain the marked deterrency from the wash-resistant formulation on cotton and nylon before washing.

The loss of deterrency on washing the nylon net with wash-resistant formulation suggests that volatile solvents are lost readily from the formulation when washed. Our failure to demonstrate deterrency with some of the insecticide formulations was unexpected.

The total number of mosquitoes killed by treated bednets is an important measure of the effectiveness of the nets, particularly as it has been shown in some studies that community-wide use of impregnated bednets can provide community-wide protection, giving a degree of protection even to those not using bednets (Magesa *et al.*, 1991).

The insecticidal ability of the ICI formulation was significantly diminished after washing, but this was not found with the wash-resistant formulations nor with the Wellcome formulation. However, one should be cautious when extrapolating this result to field trials and control programmes since the permethrin dosage varies between nets dipped into the same insecticide solution, and between samples taken from the same net.

The mortality results on nylon netting are understandable in view of the analytical data showing greater persistence of the wash-resistant and Wellcome formulations after washing. Although more permethrin persisted on cotton nets after washing, it was less insecticidal, presumably because it was concealed in crevices in the cotton fibre. This is in line with the known lower effectiveness of permethrin on cotton compared with nylon (Hossain *et al.*, 1989).

With the wash-resistant formulation, there was no sign of a masking effect of the polystyrene with the unwashed net, in contrast to the results of Miller (1990).

Exiting from the hut was the criterion used for assessing the excito-repellency (also called irritancy or enforced exophily by other workers) of the treatments that may cause mosquitoes to avoid a lethal dose. Conversely, if mosquitoes are killed quickly they may not have time to exit, so high mortality may result in apparently reduced excito-repellency.

That the unwashed untreated control net caused significantly more excito-repellency than the washed untreated control was unexpected. Textile manufacturers generally apply optical brighteners and starch to make the nets more attractive to the customer, and it is possible that these tend to repel mosquitoes and are removed by washing.

Of the unwashed nets, only the wash-resistant formulation on cotton was significantly excito-repellent. If mosquitoes are killed quickly they may not have time to exit, so low mortality, as on cotton nets, may result in greater numbers of the repelled mosquitoes reaching the exit traps. Similarly the ICI formulation, which showed poor persistence and low mortality after washing also showed higher apparent excito-repellency than when unwashed. Darriet *et al.* (1984) made a similar observation.

Without insecticide impregnation mosquitoes can probe through a net or enter holes to feed. Prevention of probing and blood-feeding is important in controlling malaria transmission and to allow the user a good night's sleep. From our results it was shown that all treated nets reduced blood-feeding and therefore reduced the chance of the net user contracting malaria. Moreover all treated nets significantly reduced the numbers of live blood-fed mosquitoes and thus reduced the chances of transmission of the disease from a net user to another person. No apparent major benefit was found from using the wash-resistant formulation compared with other formulations.. Nylon nets were more protective than cotton nets. Therefore any of these permethrin formulations would be suitable providing the dose applied is sufficiently high and the netting to which it is applied should preferably be nylon.

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