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The effect of bednets on unprotected people: open-air studies in an Afghan refugee village

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Abstract

The possibility that mosquitoes are diverted from protected people sleeping outdoors in a bednet to an unprotected person immediately adjacent was investigated during field trials conducted in an Afghan refugee village. The presence of an untreated bednet covering three people sleeping adjacent to the subject did not cause a significant rise in the biting rate on that subject. At the dose rates studied, biting rates in the presence of an Imperator® treated bednet fell slightly (although not significantly). K-Othrine® treated bednets had no discernible effect on biting rates. Similar studies conducted within giant net traps showed that the Imperator® treated bednet, and to a lesser extent the K-Othrine® treated bednet, did repel mosquitoes. Within these net traps, biting on the unprotected subject decreased considerably in the presence of the imperator® treated bednets ($P < 0.05$ for both culicines and *Anopheles stephensi* Liston in the presence of the Imperator® treated bednet). The net traps held in volatile components from the treated bednets in much the same way as the walls of a well ventilated room. We conclude that while sleeping next to a treated bednet may be of considerable benefit inside, where one sleeps in relation to a bednet outside is of little importance. Further studies revealed that for each mosquito species, insecticide induced mortality was highest when the bednet contained the preferred host of that species. This suggests that selection for pyrethroid resistance by bednets may be relatively slow to develop in areas where the primary vectors of malaria are zoophilic.

Introduction

Over the last four years HealthNet International (HNI) has been responsible for coordinating the sale of some 200,000 'family-sized' insecticide impregnated bednets (covering about 600,000 people) in refugee communities in Pakistan and in settlements in the eastern provinces of Afghanistan. In order to promote the concept of bednets for malaria control over as wide an area as possible and so as to deter reselling, HealthNet International has in the past restricted the number of bednets sold to each family. The possibility that mosquitoes (Diptera: Culicidae) are being diverted from bednet users to unprotected people sleeping nearby (Lines *et al.*, 1987) is however a matter of concern; restricted sales could result in increased biting on more vulnerable groups such as children (Curtis *et al.*, 1989).

The inhabitants of rural Afghanistan generally sleep outside during the warmer months when malaria transmission is at its height. For this reason these trials were conducted in the open air. The investigation was designed to measure both the degree of diversion caused by treated and untreated bednets and also the entomological impact of these bednets in terms of their repellent and killing effects. The trials took place in the summer of 1994.

Materials and methods

Study area

The trials were carried out at Azakhel refugee village near Peshawar, in north-west Pakistan. The numerous borrow pits, elevated water table and spring and autumn flooding in this area account for high mosquito densities throughout the summer and autumn.

The study was divided into two parts; the first was performed outside in the open air, the second was conducted inside net traps on ant free platforms. A week before the start of each experiment, family-sized bednets (length 2.4 m × width 2.2 m × height 1.8 m) made from 100 denier polyester were dipped in permethrin (Imperator® 250 g/l EC, Zeneca) or deltamethrin (K-Othrine® 25 g/l EC, AgrEvo) solution to give target doses of 0.5 gm^{-2} and 0.025 gm^{-2} respectively. During the day the bednets were sealed in plastic bags and kept indoors.

Experiment 1

This experiment was conducted at four sites spaced 50 m apart along a track bordering a field of irrigated fodder crops.

One control and three intervention scenarios were examined. At intervention sites three men (sleepers) slept together in either a deltamethrin treated, permethrin treated, or an untreated bednet. At the control site the sleepers slept without bednets. At each site a fourth man acted as the subject, lying on a mattress immediately adjacent to the sleepers. A fifth man collected mosquitoes from the subject. Workers took up their positions just before sunset. Each treatment was evaluated at each of the four sites in turn. The platforms, duties and work rotas of all staff remained constant throughout. Six four-night replicates were conducted over one month.

Experiment 2

This experiment was designed to assess the level of insecticide-induced mortality and deterrency/repellency in each situation. The protocol was identical to that above except this time the experiment was conducted under four giant net traps (length 6 m × width 5 m × height 2 m) similar to those described by Service (1993). These net traps were suspended so that their sides would form a good seal with the ground when lowered. To deny access to scavenging ants, each test site (platform) was surrounded by a water filled moat (0.2 m wide). The platforms were spaced about 10 m apart and the site was 200 m from the original test area.

Just before sunset, staff took up their positions and the sides of the traps were lowered to a distance of 0.3 m from the ground – the gap allowing free entry of wild mosquitoes. As before, mosquitoes attempting to bite the subject were collected throughout the night. In addition, a sixth man at each site collected continuously from the inside of the net trap and the sheet covered platform floor. Dead mosquitoes from the floor were placed in cups and live mosquitoes from the net traps and unprotected sleeper were placed in humidified cages. Precautions were taken to minimize the chance of mosquitoes feeding through the bednets: the bednets were securely tucked under the mattresses which were folded up to prevent the occupants pressing against the netting.

One hour before sunrise the sides of the net traps were lowered to the floor to prevent loss of mosquitoes and any remaining specimens were collected. Two hours after dawn any specimens that had died were separated from the survivors. The collections were then sorted into bloodfed and unfed, and the anophelines identified to species. An earlier experiment showed that the number of bloodfed mosquitoes entering this type of net trap from outside was negligible (Hewitt *et al.*, 1995).

As before, each treatment was evaluated at each of the four sites in turn. Twelve nights' data were collected using men as 'sleepers', and a further eight nights' data were collected using instead, a penned cow at each site. This was an attempt to boost mosquito numbers.

Statistical analysis

Data were $\log_e$ transformed where appropriate and subjected to ANOVA or a multiple range test (least significant difference method) using UNISTAT software (Toker, 1991).

Results

In the open air (experiment 1) the presence of an untreated bednet covering the three men sleeping adjacent to the subject did not cause a significant rise in the biting rate on that subject (table 1). In the presence of a permethrin treated bednet biting rates fell slightly, although not

Table 1. Summary of biting catch results. The numbers in bold are geometric means and those in italics their 95% confidence intervals. Different letters following the means indicate significantly different values. Abbreviations: NN, no bednet; C, control bednet; D, deltamethrin treated bednet; P, permethrin treated bednet.

	Culicines				*A. stephensi*				*A. nigerrimus*			
	NN	C	D	P	NN	C	D	P	NN	C	D	P
Experiment 1	**61.9** ab	**75.8** a	**60.8** ab	**48.7** b	**2.2**	**2.0**	**2.1**	**1.5**	**5.0** ab	**3.7** ab	**5.4** a	**3.1** b
(open air – men	*48.1*	*56.8*	*46.8*	*37.7*	*1.3*	*1.2*	*1.3*	*0.8*	*3.2*	*2.6*	*4.0*	*2.0*
in bednets)	*79.6*	*101.2*	*78.7*	*62.8*	*3.6*	*3.2*	*3.2*	*2.3*	*7.7*	*5.1*	*7.0*	*4.6*
Experiment 2	**36.6** a	**37.7** a	**29.0** a	**15.2** b	**6.0** ab	**7.1** a	**2.9** bc	**1.6** c	**3.1** ab	**3.6** a	**2.6** ab	**1.3** b
(net trap – men	*25.4*	*25.7*	*21.3*	*10.5*	*3.3*	*4.5*	*1.6*	*0.5*	*1.5*	*1.7*	*1.3*	*0.5*
in bednets)	*52.7*	*54.9*	*39.3*	*21.6*	*10.3*	*10.9*	*4.8*	*3.5*	*6.0*	*6.9*	*4.8*	*2.7*
Experiment (2b)	**42.0**	**43.5**	**33.3**	**41.7**	**9.9**	**10.2**	**3.9**	**4.9**	**1.6**	**2.9**	**2.5**	**1.8**
(net trap – cow	*24.1*	*35.9*	*20.1*	*8.0*	*1.4*	*2.1*	*0.1*	*1.5*	*0.1*	*1.6*	*0.0*	*0.0*
in bednets)	*72.7*	*52.5*	*55.0*	*198.6*	*48.3*	*39.0*	*21.5*	*13.0*	*5.6*	*4.8*	*11.5*	*6.5*

Table 2. Summary of net trap capture results. The numbers in bold are geometric means and those in italics their 95% confidence intervals. Different letters following the means indicate significantly different values. Abbreviations: NN, no bednet; C, control bednet; D, deltamethrin treated bednet; P, permethrin treated bednet.

	Culicines				*A. stephensi*				*A. nigerrimus*			
	NN	C	D	P	NN	C	D	P	NN	C	D	P
Experiment 2(a)	**367** a	**305** ab	**311** ab	**229** b	**32** a	**25** a	**11** b	**7** b	**24** a	**16** ab	**14** ab	**8** b
(men in bednets)	*285*	*243*	*219*	*166*	*18*	*16*	*6*	*4*	*16*	*10*	*8*	*4*
	473	*384*	*443*	*314*	*55*	*38*	*20*	*13*	*37*	*28*	*25*	*14*
Experiment 2(b)	**903**	**690**	**716**	**803**	**269** a	**121** b	**47** c	**32** c	**43** a	**27** ab	**24** ab	**18** b
(cow in bednets)	*521*	*378*	*415*	*635*	*167*	*78*	*18*	*18*	*19*	*14*	*10*	*9*
	1564	*1259*	*1237*	*1014*	*433*	*189*	*119*	*58*	*98*	*51*	*59*	*33*

significantly, for all the mosquito groups examined. Deltamethrin treated bednets had no discernible effect on biting rates.

Under the net trap (experiment 2) the untreated bednet covering the three men caused a slight increase in biting on the subject in every case although this increase was never significant. The permethrin and deltamethrin treated bednets caused reductions in biting of between 58% and 73% and 16% and 52% respectively. These reductions were significant for permethrin for the culicines and for *Anopheles stephensi* Liston (Diptera: Culicidae).

Where cows were used as 'sleepers' instead of men, no significant changes in biting rates were seen between bednets.

Net trap captures (experiment 2) were lower in the presence of an untreated bednet than in the absence of a bednet in every case; significantly lower ($P < 0.05$) in the case of *A. stephensi* and the cow (table 2). They were considerably lower in the presence of the permethrin treated bednet; significantly so ($P < 0.05$) in every case except for culicines when the bednet contained a cow. Total catches in the presence of the deltamethrin treated bednet were roughly similar to those in the presence of the untreated bednet, except in the case of *A. stephensi* where numbers were significantly lower ($P < 0.05$).

The results in fig. 1 suggest that treatment-induced trends were generally similar within each mosquito group.

Preliminary experiments with net traps revealed that almost all mosquitoes approaching a bait cow feed successfully (Hewitt & Rowland, unpublished data). A considerable proportion of the specimens collected from the net trap containing the unprotected cow in experiment 2 however were unfed. This suggests that the constant

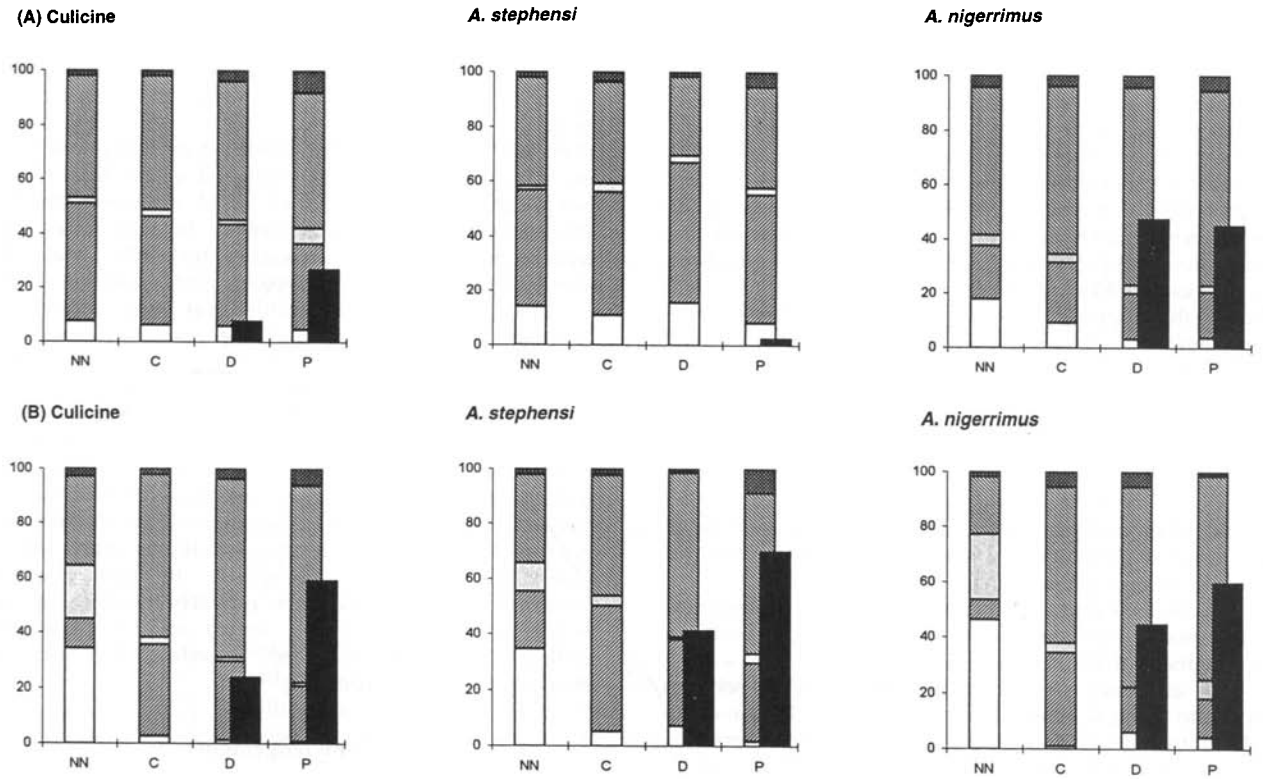

Fig. 1. Physiological condition of mosquitoes collected from net traps. (A) Men acting as 'protected sleepers', (B) cows acting as 'protected sleepers'. Abbreviations: NN, no bednet; C, control bednet; D, deltamethrin treated bednet; P, permethrin treated bednet. Shading (from bottom to top): white, live engorged; hatched, live unengorged; stippled, dead engorged; cross-hatched, dead unengorged; dark stippled, knocked down; black bars, insecticide induced mortality.

collecting from the inside of the net trap removed many mosquitoes before they had a chance to reach the bait; it is well documented that many species of mosquito rest on vegetation and other convenient surfaces such as net traps immediately prior to biting a host (Colless, 1959; Moorhouse & Wharton, 1965). The high proportion of unfed mosquitoes captured in each net trap is probably therefore an artefact of the experimental design and this should be taken into account when interpreting fig. 1.

A comparison of the bar representing the 'no-net control' (NN) for each mosquito group in the man baited bednets with the corresponding bar for each group in the cow baited bednets, illustrates the relative ease with which mosquitoes feed on cattle. Single factor ANOVA revealed that these differences were highly significant for all the mosquito groups: *A. stephensi*, $P = 0.001$ ($F_{(1,14)} = 16.91$); *A. nigerrimus* Giles, $P = 0.001$ ($F_{(1,14)} = 19.87$); culicines, $P = 0.002$ ($F_{(1,14)} = 26.20$).

The dark bars (fig. 1) represent the level of insecticide-induced mortality (Abbott's correction) found in each net trap containing a treated bednet. Mortality rates were considerably higher when the treated bednets were baited with cows than when they were baited with men for both *A. stephensi* ($P = 0.015$, $F_{(1,30)} = 6.70$) and for culicines ($P = 0.014$, $F_{(1,30)} = 6.81$). For *A. nigerrimus* mortality rates were similar in the presence of cows and men.

Discussion

The first experiment was designed to measure directly any diversion of mosquitoes from people sleeping in bednets to unprotected people in the vicinity. Even in the rather extreme scenario examined, where one unprotected person was sleeping immediately adjacent to three people in a bednet, there was little evidence of diversion. There was no significant increase in biting in the presence of the untreated bednet (although numbers of biting culicines were up by 20% overall) and no significant decrease in the presence of either of the pyrethroids tested (although in the presence of the permethrin treated bednet, biting rates fell for all the mosquito groups examined). These findings are supported by the results of recent clinical studies in the region which showed that unprotected people sleeping outside in the same compound as bednet users were no less likely to contract malaria than those sleeping in compounds without bednet users (Rowland *et al.*, 1996).

The reductions in biting rates in the presence of treated bednets seen in the net trap experiments suggest that the net trap was holding in volatile repellent components from the insecticides or their formulations (Lindsay *et al.*, 1991); indeed, when the bednets were freshly dipped a strong smell was apparent on entering the net trap. One would expect this effect to be even more pronounced inside huts.

We conclude that while it may be beneficial to sleep next to a treated bednet when inside, where one sleeps in relation to a bednet outside is of little importance.

The efficiency of net traps varies greatly between mosquito groups: experiments in which single, pre-dawn collections were made showed that the technique captured 85% of culicines but only 11% of *A. stephensi* (Hewitt *et al.*, 1995). On the basis of these findings, one can assume that the all-night catches performed during this current investigation resulted in the capture of virtually all the culicines that entered the trap.

When men were used as sleepers, culicine net trap catches were 17% lower in the presence of an untreated bednet than when there was no bednet ($P > 0.05$). This reduction can be attributed to the diminished attractiveness of the bednet-covered bait. The permethrin treated bednet caused an additional 25% reduction in the culicine net trap catch suggesting that mosquitoes are deterred from entering the net trap; again probably a response to formulation volatiles. The deltamethrin treated bednet did not cause any reduction in the size of the culicine catch. For culicines at cow-baited bednets there were no clear differences between treatments; the powerful stimuli coming from the cow presumably overcame all other influences.

Despite the inefficiency of net traps for anopheline capture, the technique did reveal similar trends to those seen with culicines. When men were used as sleepers the permethrin treated bednet caused a 72% reduction in the size of the *A. stephensi* catch compared with the untreated bednet, while deltamethrin caused a 56% reduction.

Hut trials in Tanzania revealed similar reductions in catch size with untreated bednets (Lines *et al.*, 1987). There are two possible explanations: bednets may physically restrict the odour plume or (and), by preventing mosquitoes from feeding, they may prevent the release of aggregation pheromones. Recent studies, conducted by Alekseev, Rasnitsyn and Vitilin, and by Ahmadi and McClelland, demonstrated an 'invitational effect' whereby female mosquitoes (*Aedes communis* (De Geer) and *A. sierrensis* (Ludlow)) are attracted to a host in proportion to the numbers of females already feeding on that host (referred to in Service, 1993). Either way, theoretically this effect should result in increased biting on unprotected people sleeping further afield. In Pakistan however, vectors are zoophilic and livestock is abundant and it therefore seems unlikely that this type of diversion would have a significant impact on malaria incidence.

For *A. stephensi*, insecticide-induced mortality was very much higher when bednets contained a cow than when they contained men. In contrast, for *A. nigerrimus* insecticide-induced mortality was similar in both cases. *A. stephensi* has a marked preference for cattle, while *A. nigerrimus* (which prefers to feed on goats) does not show any strong preference for cattle over men (Hewitt & Rowland, unpublished data). These results suggest that the community impact of an impregnated bednet intervention will depend on the host preferences of the target mosquito and the availability of alternative hosts: while treated bednets may have a marked impact on parity rates of anthropophilic vectors such as *A. gambiae* Giles they are unlikely to have much impact on highly zoophilic vector species such as *A. stephensi*. This implies that selection for pyrethroid resistance by bednets would be much lower where the vectors are zoophilic. In such regions pyrethroid impregnated bednets probably therefore offer a longer term malaria control option and so particular caution should be exercised regarding the use of pyrethroids for wall spraying.

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