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Permethrin-treated bed nets do not have a 'mass-killing effect' on village populations of *Anopheles gambiae s.l.* in The Gambia

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Abstract

In The Gambia, the use of permethrin-treated bed nets has led to a reduction in morbidity and mortality from malaria in children. However, no clear evidence has been found for a 'mass-killing effect' on the mosquito vectors as a result of this intervention. Two further entomological studies to investigate this phenomenon have been carried out. In one study, 20 villages were paired so that bed nets in one member of each pair were treated with permethrin. In the other, a cross-over design was used in which treated and untreated bed nets were exchanged between 2 villages. Longevity, biting rate and resting density of the malaria vector population and sporozoite rates were assessed in both studies. Malaria vectors were equally abundant and long-lived, and as likely to be infective, in villages with treated bed nets as in those with untreated nets. However, a clear reduction in the density of the indoor-resting population of mosquitoes in rooms with treated bed nets was found, probably reflecting the excito–repellency of the insecticide. This study confirmed that, in The Gambia, the protection against death and morbidity from malaria seen in children using treated bed nets must be due primarily to personal protection rather than to a 'mass-killing effect' on the mosquito vector population at a village level.

Keywords: malaria, *Plasmodium falciparum*, *Anopheles gambiae*, vector control, permethrin, bed nets, children, The Gambia

Introduction

A major reduction in the vectorial capacity of the vector population is the most desirable effect of using an insecticide for malaria control. Insecticide-treated bed nets may be able to provide this as well as giving personal protection. Some studies of insecticide-treated bed nets have shown a 'mass-killing effect' with reductions in survivorship, sporozoite rate and density of the mosquito vector population at a village level (MAGESA *et al.*, 1991; KARCH *et al.*, 1993), or a dramatic reduction in biting density (KERE *et al.*, 1993; JANA-KARA *et al.*, 1995). In other situations, little evidence for such an effect has been seen (CHARLWOOD & GRAVES, 1987; SOMBOON *et al.*, 1995).

In The Gambia, initial epidemiological studies suggested that malaria protection improved as coverage with impregnated bed nets increased. When individual bed nets were treated with permethrin or placebo, and used by less than 10% of the children in one village, those who slept under treated bed nets had fewer clinical episodes of malaria than control children but other features of malaria infection, such as splenomegaly, parasitaemia and packed cell volume (PCV), were not altered significantly (SNOW *et al.*, 1987a). In a subsequent community-level study involving 16 villages, in 7 of which all bed nets were treated, a significantly lower acquired spleen rate and a significantly higher mean PCV were found in children in the treated villages, in addition to a reduction in the number of clinical cases (SNOW *et al.*, 1988). This apparent improvement in malaria protection when the coverage of impregnated bed nets increased is consistent with a mass-killing effect on the vector population.

Entomological observations in both studies involved collections of indoor resting mosquitoes (collected in bed nets and by knock-down pyrethrum spray catches [PSC]) and exit trap collections (ETC). The results of the first study showed significantly fewer females resting in bed nets, fewer unfed females resting in treated rooms, and a higher percentage of females exiting from treated rooms (SNOW *et al.*, 1987b) measured as the ratio ETC/ETC+PSC. In the second study, when the treatment of bed nets was undertaken at a village level, collections were again carried out in treated rooms in treated villages and in untreated rooms in untreated villages and, this time, parous rates, the human blood index (HBI) and sporozoite rate were also evaluated. As in the previous study, there was a significant reduction in the number of fed females resting in rooms with a treated bed net. The reduction in human–vector contact was estimated to be over 90%. However, this calculation was based on the number of fed females of *Anopheles gambiae s.l.* collected inside, and exiting from, treated and untreated rooms, together with their HBI (LINDSAY *et al.*, 1989). This estimate assumed that all mosquitoes that entered and fed in a room were accounted for, while it is likely that only a fraction of exiting females were caught in the traps, the others leaving through eaves, doors etc. In this way a bias in the estimated efficacy of the treated bed nets may have been introduced by the excito–repellent effect of the treated nets. No significant difference was found in parous rates, sporozoite rates or the HBI between treated and untreated villages.

In a subsequent study (LINDSAY *et al.*, 1993), also conducted on a villages scale, untreated ('sentinel') rooms in treated villages were used for entomological collections. The use of sentinel rooms avoided the above-mentioned bias caused by the excito–repellent effect of the insecticide when spray catches and exit traps are used. Little evidence for a mass-killing effect was found; there was no indication that treated bed nets caused a decrease in density, measured by light traps and spray catches, and the HBI and sporozoite rates were similar in treated and in untreated villages. Reductions in the sporozoite rate and in mosquito numbers were found in treated villages after the intervention, but these changes were noted also in villages without treated bed nets. However, the possibility of a mass killing effect could not be ruled out completely because movements of mosquitoes between treated and neighbouring untreated villages might have masked the mass killing effect of the insecticide, thus accounting for an overall reduction in mosquito numbers. Moreover, in the post-intervention year the rainfall was lower than in the previous year, and this could also have accounted for a reduction in mosquito numbers.

Knowledge of whether treated bed nets can be expected to produce a mass-killing effect at the village level under local conditions is important for the planning

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and implementation of malaria control programmes. If a mass killing effect occurs then, as with DDT house spraying, programmes encouraging high coverage are likely to be most effective. On the other hand, if only individual protection is given by the use of treated bed nets, different strategies could be employed, for example encouraging target groups especially at risk for malaria to acquire and impregnate their bed nets.

In order to clarify whether or not permethrin-impregnated bed nets have a mass killing effect in The Gambia, 2 further entomological studies were carried out. The first involved a survey of 10 treated and 10 untreated villages, and the second was a 'cross-over' study in 2 villages. Longevity, biting rate, resting density and sporozoite rates were assessed.

Materials and Methods

Village survey

Collections were carried out in villages in the central area of The Gambia, between 200 and 280 km from the Atlantic coast, in July and October 1992. Twenty primary health care (PHC) villages (see footnote to Table 1) were selected from amongst those used for epidemiological evaluation of the Gambian National Impregnated Bednet Programme (NIBP) (D'ALESSANDRO *et al.*, 1995). Ten of these villages had bed nets that had been treated between 10 June and 10 July 1992, with permethrin at a target dose of 250 mg/m^2. Pairs of treated and untreated villages were matched by proximity.

A single human-landing collection was carried out in each village between 9 and 30 July 1992. Two teams of 8 collectors were recruited from villages in the study area. One Medical Research Council (MRC) supervisor was assigned to each team. One team worked in a house in a treated village while the other worked on the same night in a house in its paired untreated village. The collectors worked in pairs, 2 indoors and 2 outdoors, for consecutive periods of 3 h, from 19:00 to 07:00. They exchanged places every hour. After 3 h they were relieved by the other 4 collectors, who worked in the same way. All mosquitoes landing on them were collected and placed in a paper cup with a pad of sugar solution.

On the morning after human-landing collections had been done, pyrethrum spray catches were carried out using an aerosol of 0·3% d-allethrin and 0·1% d-phenothrin (Target®, Reckitt & Colman) in 5 rooms in each village. The teams worked alternately in treated and untreated villages. In addition, in the same 10 pairs of villages, mosquitoes were collected in 4 exit traps set on one night in each village during October 1992.

Unfed female mosquitoes of the *A. gambiae* complex collected in human-landing catches and in exit traps were dissected for parity determination from the ovarian tracheoles. To avoid possible bias, the slides for parity determination were coded and scored without knowledge of the village of collection.

Heads and thoraces of *A. gambiae s.l.* mosquitoes were examined by enzyme-linked immunosorbent assay (ELISA) (WIRTZ *et al.*, 1987) to determine the presence of *Plasmodium falciparum* circumsporozoite antigen. Cytogenetic examination was carried out to identify the species of a sample of *A. gambiae s.l.* collected in the study area (COLUZZI *et al.*, 1979).

Cross-over study

The cross-over study was carried out in 2 villages, Madina and Jakoto, in Niamina District, 180 km from the coast, in October 1993. The main objective of this study was to determine changes in the gonotrophic cycle length using mark–release recapture, the results of which have been reported elsewhere (QUIÑONES *et al.*, 1997). However, the sampling routines (spray catches and exit traps) also allowed measurements of changes in density, parous rates and sporozoite rates, and these results are presented here.

At the beginning of the study, the objective and procedures of the study were explained to the residents in each village and, through the elders, their consent was obtained. The people in Madina were given new permethrin-treated bed nets, one for each bed in the village. Bed nets, made of light cotton netting, were purchased locally, and dipped in permethrin, target dose 500 mg/m^2, at the MRC field station in Farafenni to avoid contamination of mattresses as far as possible. The people in Madina were asked to use the new, treated bed nets for 2 weeks but to keep their own untreated ones in a safe place. In the other village, Jakoto, people were asked to continue using their own bed nets, which were untreated. Mosquitoes were collected in both villages for 11 d using pyrethrum spray catches in 10 rooms every day. Five exit traps were set daily in each village. After 2 weeks, the treated bed nets were collected from Madina and the people began using their own untreated nets again. The people in Jakoto were then provided with new bed nets, which were similarly dipped in the village, and dried on the mattresses. People were asked to use them instead of their own untreated bed nets. The collection of mosquitoes continued for another 2 weeks as before. At the end of the study, permethrin-treated bed nets were given to both villages.

Table 1. Numbers of female *Anopheles gambiae s.l.* collected in human-landing collections and number per room resting indoors obtained using pyrethrum spray catches in pairs of villages with insecticide-treated or untreated bed nets

			Villages with			
			Treated nets		Untreated nets	
Date (July 1992)	Village pair[a]	Days after insecticide treatment	Human-landing[b]	Pyrethrum spray[c]	Human-landing[b]	Pyrethrum spray[c]
9	1	21	470	27·20 (5)	737	114·96 (5)
10	2	10	150	16·80 (4)	31	26·49 (4)
15	3	24	210	5·00 (5)	84	17·96 (5)
17	4	8	77	2·63 (3)	38	8·20 (5)
21	5	30	44	4·26 (5)	57	6·09 (5)
22	6	14	44	1·98 (5)	140	9·50 (5)
24	7	32	112	8·45 (5)	155	18·26 (5)
28	8	34	28	2·10 (5)	29	7·70 (5)
29	9	20	13	1·99 (5)	15	1·05 (5)
31	10	20	5	2·57 (5)	7	2·46 (5)
Total	–	–	1153	4·91 (47)	1293	10·52 (49)

[a]Pairs of villages in The Gambia (with and without treated bed nets) matched by proximity: 1, Jafaye–Sotokoi; 2, Jahally–Saruja; 3, Kudang–Mbien; 4, Madina Nfally–Taifa; 5, Pacharr–Kerewan; 6, Brikamaba–Tabananeh; 7, Mbaien Maka–Mamufana; 8, Pathe Same–Batinjol; 9, Sare Futa–Fula Bantang; 10, Fas Abdou–Boweram.
[b]Total number caught, 19:00–07:00, indoors and outside.
[c]Geometric mean no. caught per room (no. of rooms in parentheses).

Unfed mosquitoes were dissected for parity determination every day and the percentage with circumsporozoite antigen in heads and thoraces was determined by ELISA. Palps of a sample of *A. gambiae s.l.* were dissected and measured for species determination from the palpal ratio (COLUZZI, 1964). Females with a palpal ratio of 0·81 or more were classified as *A. melas* and those with a lower ratio as fresh-water *A. gambiae* (see BRYAN, 1980).

Results

Village survey

Human-landing collections. The total number of *A. gambiae s.l.* collected by human-landing collections in treated villages (Table 1) was correlated with that in the corresponding untreated village (r=0·899, t=5·8, d.f.=8, P<0·001), thus validating the paired design. The difference between treated and untreated villages in the total number of *A. gambiae s.l.* collected in human-landing collections was not significant in a paired t test after log-transformation of the numbers collected (t=0·19, d.f.=9, P=0·848) (Table 1). Significantly fewer *A. gambiae s.l.* were found resting in rooms with treated bed nets (geometric mean 4·91/room) than in rooms with untreated bed nets (geometric mean 10·52/room) (Wilcoxon signed rank test with geometric means by pair of villages, P=0·009) (Table 1). Since no such difference was seen in human-landing collections, this was presumably due to the excito–repellency effect of permethrin. There was no significant difference between village groups in the proportions of females in each gonotrophic stage (unfed, fed, gravid) of *A. gambiae s.l.* resting indoors in the morning (Table 2). The number of unfed mosquitoes resting indoors was very low in both treated and untreated villages.

Parous rates. Parous rates from the samples collected in human-landing catches (one month after bed net treatment) were very variable in both treated and untreated villages (Table 3). In some cases the sample sizes were very small. No effect of the treatment was observed on parous rates, which were not reduced in villages with treated bed nets. A Mantel–Haenszel [M–H] χ^2 test showed the opposite, significantly higher parous rates in villages with treated bed nets than in those with untreated nets (M–H χ^2=9·93, P=0·001) (Table 3). In the samples collected in exit traps, parous rates were also variable. However, the parous rates of *A. gambiae s.l.* collected in exit traps 4 months after treatment were significantly lower in treated than in untreated villages (M–H χ^2=8·84, P=0·003) (Table 4).

Sporozoite rates. Two of 640 (0·31%) and 11 of 1344 (0·82%) mosquitoes were found to be positive by ELISA from the treated and untreated villages, respectively. This difference was not statistically significant (Fisher's exact test, P=0·245).

Cross-over study

Exit trap collections. The numbers of *A. gambiae s.l.* collected in exit traps in the presence of treated and untreated bed nets is shown in the Figure (A). A paired t test on log-transformed data showed that the difference in density was not significant (t=1·253, d.f.=18, P=0·226). The same result was obtained when the test was repeated omitting the data from the period when the traps were probably contaminated with insecticide (see below).

Table 2. Indoor resting collections of female *Anopheles gambiae s.l.* classified by gonotrophic stage in pairs of villages with insecticide-treated or untreated bed nets

		Villages with							
Date	Village	Treated nets				Untreated nets			
(July 1992)	pair[a]	Unfed[b]	Fed[b]	Gravid[b]	Total	Unfed[b]	Fed[b]	Gravid[b]	Total
9	1	43·9	55·5	0·7	155	39·8	58·9	12·4	645
10	2	3·7	72·9	23·4	107	3·6	82·6	13·4	138
15	3	0	74·2	25·8	31	1·1	89·3	9·7	93
17	4	0	100	0	10	1·3	17·9	80·8	78
21	5	0	86·7	13·3	30	0	71·7	28·3	46
22	6	0	82·8	17·2	29	0	82·2	17·8	73
24	7	0	78·6	21·4	56	3·0	79·0	18·0	100
28	8	0	69·2	30·8	39	0	57·3	42·7	82
29	9	0	100	0	11	12·5	75·0	12·5	8
31	10	0	72·2	27·8	18	0	83·3	16·7	18
Total	–	14·8	70·4	14·8	486	20·9	64·9	14·2	1281

[a]See footnote to Table 1.
[b]Expressed as a percentage of the total female catch per village. Wilcoxon's signed rank test comparing the proportions in villages with treated and untreated nets gave the following results: unfed, P=0·44; fed, P=0·50; gravid, P=0·50.

Table 3. Parous rates of *Anopheles gambiae s.l.* from human-landing collections in pairs of villages with insecticide-treated or untreated bed nets

		Villages with			
Date	Village	Treated nets		Untreated nets	
(July 1992)	pair[a]	Parous (%)	No. examined	Parous (%)	No. examined
9	1	69·23	104	35·71	98
10	2	91·25	80	72·72	22
15	3	66·31	95	71·15	52
16	4	86·48	37	60·00	30
20	5	80·00	35	95·55	45
21	6	100·00	21	70·17	114
23	7	31·31	99	37·30	126
27	8	56·52	23	65·21	23
28	9	100·00	8	66·00	12
30	10	33·33	6	50·00	2
Overall[b]	–	76·44	508	62·38	524

[a]See footnote to Table 1.
[b]Weighted mean or total.

The number of females collected in exit traps was very similar in both villages during the first part of the study. However, soon after the exchange of nets, exit trap densities declined in the treated but not in the untreated village, suggesting that the traps had been contaminated during the dipping process. They were therefore washed 3 d after the cross-over and the number of mosquitoes collected in traps in the treated village, Jakoto, increased again.

As in the village study, the numbers of mosquitoes collected by morning pyrethrum spray catches were lower when treated bed nets were present than when they were absent. When bed nets were treated, the number of *A. gambiae s.l.* collected resting indoors was, on average, one-quarter of that found when the nets were untreated. This change was seen in both villages (Figure, B). A paired t test showed the difference to be significant (t=8·574, d.f.=17, P=<0·001).

Table 4. Parous rates of *Anopheles gambiae s.l.* from exit trap collections in pairs of villages with insecticide-treated or untreated bed nets

		Villages with			
Date	Village	Treated nets		Untreated nets	
(Sep.–Oct. 1992)	pair[a]	Parous (%)	No. examined	Parous (%)	No. examined
29	5	64·15	53	73·47	49
30	1	37·27	110	54·72	106
1	2	74·36	78	72·88	59
2	4	80·00	10	87·18	39
3	7	23·81	21	60·00	35
5	8	24·14	58	45·45	11
6	3	40·43	94	61·76	34
7	6	53·85	104	75·68	74
8	9	33·33	6	53·85	13
13	9	94·12	119	72·12	104
15	7	71·74	46	69·23	13
16	4	76·67	30	90·00	30
Overall[b]	–	56·15	729	68·03	567

[a]See footnote to Table 1.
[b]Weighted mean or total.

Table. 5. Sporozoite rates of *Anopheles gambiae s.l.* in the cross-over study, October 1993

		No. of mosquitoes				
Village	Treated[a]	Tested	Positive	Rate	χ^2	P
Madina	Yes	2376	24	0·0101	0·21	0·649
Jakoto	No	2376	20	0·0084		
Madina	No	2534	14	0·0055	0·03	0·989
Jakoto	Yes	2546	15	0·0059		
Madina	Yes	4922	39	0·0079	0·21	0·646
Jakoto	No	4910	34	0·0069		

[a]Insecticide treatment of bed nets.

Parous rates. The parous rates observed in the cross-over study are shown in the Figure (C). A similar pattern was seen in the presence of treated and untreated bed nets throughout the study, although greater variation was found in the second part of the sampling period. There was no significant difference between parous rates in the presence of treated and untreated bed nets (M–H χ^2=1·24, P=0·26).

Sporozoite rates. Sporozoite rates were not significantly different in the presence of treated and untreated bed nets (Table 5).

Species identification

In the village survey, 96·1% of 154 *A. gambiae s.l.* were characterized cytogenetically as *A. gambiae s.s.* savannah type (COLUZZI *et al.*, 1985), the remaining 3·9% being *A. arabiensis*. In the cross-over study, according to the palpal ratio, 82·4% of 321 females caught in the 2 study villages were freshwater *A. gambiae s.l.* (either *A. gambiae s.s* or *A. arabiensis*), while 17·6% were identified as *A. melas*, the saltwater sibling species.

Discussion

The findings presented in this paper illustrate 2 different approaches to the study of mosquito populations in villages with treated and untreated bed nets. In the first, single observations were made in as many replicate pairs of villages as possible during a short period of time. In the second, repeated observations were made in 2 villages in a cross-over design, which allowed for differences over time.

The paired village study should have demonstrated conclusively whether or not there were consistent differences in density, parous rate or sporozoite rates between the villages with treated and untreated bed nets and was large enough to have detected a two-fold difference in density and parous rates, as found in Burkina Faso (ROBERT & CARNEVALE, 1991) and Zaire (KARCH *et al.*, 1993). For logistic reasons, only a single observation was made in each pair of villages but, because of the large sample size, this should not have perturbed the results.

The cross-over study was a more sensitive test because local differences between the villages were controlled for by the design of the study. One possible source of bias with this design is the carry-over effect of the insecticide-treated bed nets and contamination of rooms with insecticide. A gap of some days between the cross-over of treated bed nets was used in an attempt to overcome any possible carry-over effect. As in the paired village study, no evidence of a mass killing effect was found. No significant difference was observed in any of the features measured (density, parous rate or sporozoite rate) during periods when treated bed nets were used and when they were not.

Both studies covered a relatively short period of time and were carried out soon after the impregnation of the bed nets. Therefore, only immediate changes in the vector population could be evaluated and it was not possible to measure long term effects. Expected immediate changes include a reduction in longevity of the popula-

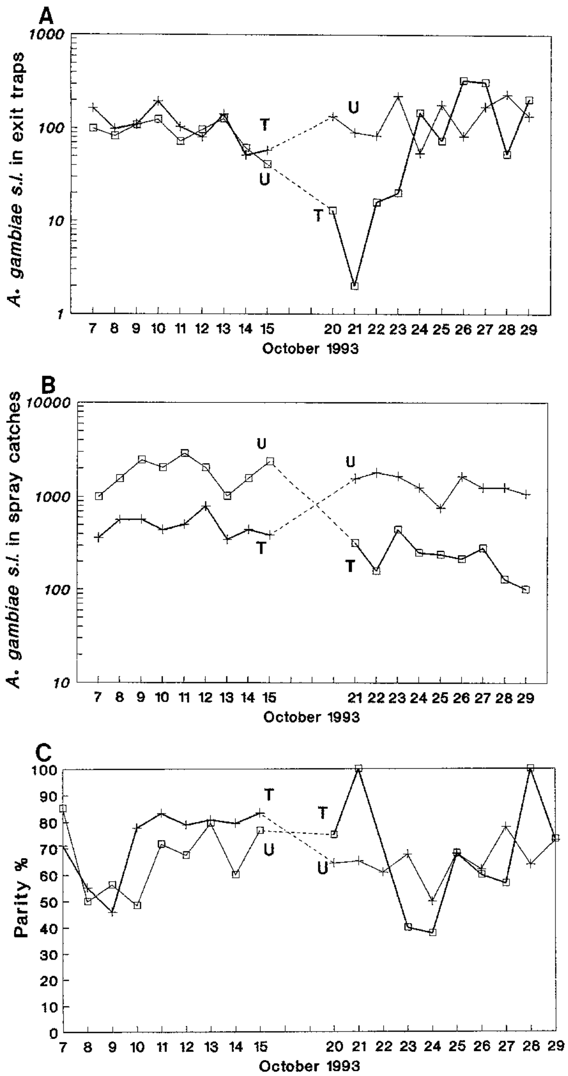

Figure. *Anopheles gambiae s.l.* in Madina (+) and Jakoto (□), The Gambia. Numbers of females collected in exit traps (A) and resting indoors (B), and the parous rate (C), in the presence of insecticide-treated (T, heavy line) and untreated (U, light line) bed nets.

tion due to mass killing, as demonstrated clearly in Tanzania (MAGESA *et al.*, 1991). This was not observed in the present studies.

The absence of a mass killing effect following the use of insecticide-treated bed nets has been reported in Thailand (SOMBOON *et al.*, 1995) and has been explained by the exophagic, zoophilic and early evening biting habits of the vectors in that country. However, in The Gambia *A. gambiae s.l.* is endophagic, anthropophilic and late biting. Furthermore, in other countries with the same malaria vector (i.e., Tanzania, Zaire and Burkina Faso) a mass killing effect has been found. How can the difference between The Gambia and the other countries with the same mosquito vector be explained?

One possibility is that the effect was masked by movements of mosquitoes between treated and untreated villages, as proposed by LINDSAY *et al.* (1993). Limited evidence for a mass killing effect has been obtained from one isolated village in The Gambia (THOMSON *et al.*, 1995a). A mark–release recapture experiment has shown that movements of mosquitoes between villages do take place in The Gambia. The proportion of mosquitoes in one village that came from a neighbouring village 1–1·5 km away was calculated to be 17·2% (95% confidence interval [95% CI] 12·2–22·4) for blood-fed females released from bed net collections and 20·1% (95% CI 14·7–25·3) for releases of unfed females from exit trap collections (THOMSON *et al.*, 1995b). In Tanzania, in a similar mark–release recapture experiment between villages separated by a similar distance, only 1·5% (3/204) of recaptures were in a village other than that of release (NJUNWA, 1993), while this proportion in The Gambia was 26·5% (98/370) (THOMSON *et al.*, 1995b). This difference between The Gambia and Tanzania could be due to differences in the distribution of breeding places in relation to villages. In Tanzania, the most common breeding places are small puddles, streams and rice fields within and near villages (K. Njunwa, personal communication), while in The Gambia it is difficult to find breeding places in the villages and the main breeding places are usually swamps along the river. These swamps are often situated between villages, so that female mosquitoes, having oviposited, may look for their next blood meal in a village other than the one in which they took their previous meal.

Another possible explanation for the absence of a mass killing effect in The Gambia is that mosquitoes are not generally killed by treated bed nets in The Gambia, perhaps because the material of the nets in The Gambia is different from that used both in previous experimental hut studies in which killing of mosquitoes has been measured (LINDSAY *et al.*, 1991; MILLER *et al.*, 1991) and in countries where a mass killing effect following insecticide impregnation of bed nets at a community level does occur (MAGESA *et al.*, 1991; KARCH *et al.*, 1993). In The Gambia, synthetic sheeting, synthetic netting and cotton sheeting are the most common materials for bed nets (AIKINS *et al.*, 1993), while in the experimental hut studies and in the village trials in Tanzania, for example, standard polyester bed nets have been used. In a recent experimental hut trial, low mortality was found when Gambian bed nets were used (2 nylon netting, 2 synthetic sheeting and one synthetic muslin netting) compared with previous results using the same experimental huts with imported polyester bed nets (L. C. S. Nagle, unpublished observations).

Although the results of resistance tests confirm the susceptibility of the different populations of *A. gambiae s.l.* (M. Jawara, unpublished data), the possibility that the vector in The Gambia may be slightly more tolerant to permethrin than populations in other countries cannot be completely ruled out.

This and previous studies (LINDSAY *et al.*, 1993; THOMSON *et al.*, 1995a) confirm that, in The Gambia, the protection against malaria seen in children using treated bed nets must be attributed to personal protection rather than to a mass killing effect at village level. Individual use of treated bed nets was recorded in the epidemiological evaluation of the NIBP and the protective effect was confirmed to be associated, at least in part, with personal protection (D'ALESSANDRO *et al.*, 1995) rather than being a community effect.

The occurrence of individual protection by permethrin-treated bed nets and the absence of a mass killing effect at community level makes it justifiable to direct intervention programmes to high risk target groups such as children and pregnant women. However, it is important to clarify how mosquitoes are managing to feed and survive in villages with treated nets. If mosquitoes are biting animals instead of protected people, there is no reason not to promote individual acquisition of a treated bed net as the strategy for implementation of this control measure. However, if diversion to unprotected people is occurring, for example diversion from protected children to adults, a strategy encouraging mass use of impregnated bed nets is required.

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