A malaria control trial using insecticide-treated bed nets and targeted chemoprophylaxis in a rural area of The Gambia, West Africa

7. Impact of permethrin-impregnated bed nets on malaria vectors

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Abstract

The impact of permethrin-impregnated bed nets on malaria vectors was studied in 6 pairs of villages during the rainy season in 1989. In each pair, the residents of one village had their nets treated whilst those of the other remained untreated. Routine collections of mosquitoes were made outdoors in the early evening using human-biting collections, and indoors with insecticide sprays, light traps and by searches under bed nets. Mosquitoes of the *Anopheles gambiae* complex, *An. gambiae sensu stricto*, *An. arabiensis* and *An. melas*, were present in large numbers for 5 months of the study period. These mosquitoes were susceptible to permethrin as judged by bioassay results. Outdoor human-biting rates in the early evening in communities with treated bed nets were similar to those in communities with untreated nets. In villages with treated bed nets most biting occurred outdoors in the early evening with little taking place under impregnated nets. The insecticidal activity of permethrin-impregnated bed nets, dipped by the local population, provided good individual protection against mosquitoes throughout the rainy season and bed nets remained effective even when washed up to 3 times. There was little to suggest that the use of insecticide-treated nets reduced the survival of mosquito populations in villages with impregnated nets. The absence of the expected village-wide effects of net impregnation may have resulted from the circulation of mosquitoes between villages with treated and untreated nets. The proportion of mosquitoes which fed on humans did not differ significantly between villages with treated and untreated nets. Permethrin-impregnated bed nets proved an effective barrier against vectors when people were under their nets, but had no apparent effect on biting outdoors before individuals retired to bed.

Introduction

Sleeping under a permethrin-impregnated bed net is a highly effective means of protection from bites of night-biting mosquitoes. The insecticide formulation used for treating nets can deter mosquitoes from entering houses and cause mosquitoes landing on treated netting to be killed or irritated and repelled (DARRIET *et al.*, 1984; LINES *et al.*, 1987; ROZENDAAL, 1989; MILLER *et al.*, 1991; LINDSAY *et al.*, 1992a). Trials of insecticide-treated bed nets in many parts of the tropics have shown that this form of personal protection operates well against several different malaria vectors. Moreover, the degree of protection afforded to a community can be enhanced by using insecticide-impregnated bed nets on a large scale (CURTIS *et al.*, 1990; MAGESA *et al.*, 1991). In such circumstances, the high mortality among mosquitoes attracted to these nets can cause a reduction in the population of mosquitoes in a village and in the mean age and proportion of sporozoite-bearing mosquitoes. This malaria control strategy has been effective in reducing morbidity in areas of relatively low transmission (SNOW *et al.*, 1988; CURTIS *et al.*, 1990) but was less successful in places where transmission was more intense (ROZENDAAL, 1989; CURTIS *et al.*, 1990; WHO, 1990).

The present study describes a series of entomological investigations designed to assess the effects of permethrin-treated bed nets in an area of seasonal malaria transmission in The Gambia. A description of the malaria vectors in this area during the rainy season immediately before this intervention has been given by LINDSAY *et al.* (1993). The study population consisted of 21 157 people in 73 villages in a rural area on the south bank of the River Gambia. Bed nets in 17 villages were impregnated with a pyrethroid insecticide, permethrin, whilst the remaining communities had untreated nets and served as controls. In addition, half of the children aged 6 months to 5 years in villages with treated nets were given chemoprophylaxis with Maloprim® weekly. The other half received a placebo. This intervention resulted in a 63% reduction in mortality in children aged 1–4 years (ALONSO *et al.*, 1993c). This protection against mortality was probably a result of using insecticide-treated nets as there was no significant additional effect when children were given weekly chemoprophylaxis.

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Materials and Methods

Study design

Descriptions of the study area, the adult vector populations in the villages and the implementation of the control programme have been described elsewhere (ALONSO *et al.*, 1993a, 1993b; LINDSAY *et al.*, 1993). Entomological investigations were carried out in 6 pairs of villages: Jessadi and Barokanda, Katamina and Male Kunda, Pakali Ba and Madina, Dasilami and Niawoorulung, Dongoro Ba and Wellingara Ba, and Jalangbereh and Sitahuma. The first named village in each pair of settlements was a primary health care (PHC) village, where basic health care was provided by a village health worker and a traditional birth attendant. During the intervention year, most people in these villages slept under bed nets treated with permethrin (25% emulsifiable concentrate, ICI Public Health) at a target dose of 0·5 g/m^2 (the actual mean permethrin dose was 0·2 g/m^2; ALONSO *et al.*, 1993b). An adjacent village without health care facilities (non-PHC village), where residents slept under untreated nets, served as a control for each intervention village.

Environmental studies

Meterological recordings were made using standard methods (METEROLOGICAL OFFICE, 1982) at Pakali Ba throughout the study.

The salinity of water collected from the River Gambia was measured using a refractometer (Aguafauna®, Chemlab Scientific Products). One litre samples of water were collected along the river and from a major tributary, the Sofaniama Bolon, at Bai Tenda, Baro Kunda-Tenda, Pakali Ba, Sambang Tenda, Kani Kunda Wharf Town, Balanghar Wharf Town and Jessadi Wharf Town. Collections were made close to the bank at the time of the highest tide at the end of the rainy season during the period of maximum mosquito production.

Entomological surveillance

Routine mosquito catches were made using 4 different sampling techniques: human-biting catches, light trap collections, 'knock-down' sampling, and bed net searches, from June until the end of December 1989. Four men were employed as collectors in each of the 12 villages. These men received weekly chloroquine prophylaxis and slept under an untreated bed net in a room on their own, in a separate quarter of each village. Each man collected mosquitoes outdoors between 19:30 and 23:00 on one evening each week. Each person sat alone outside his house and collected insects landing on his exposed limbs using an aspirator and torchlight. Further collections were made indoors in the bedroom of each collector using a CDC light trap on one night each week and with an aerosol of insecticide on another night, as described elsewhere (LINDSAY *et al.*, 1993).

Twenty bed nets were selected at random from each PHC village (n=120) in June 1989 and sampled at weekly intervals until the end of the study. Nets were searched between 07:00 and 10:00 with data being used in the analysis only if the net was found with both entry flaps closed. Bed nets were marked with a water-soluble pen and inspected each week for evidence of washing. Washed nets were identified by the disappearance of the ink label and were marked again after each inspection.

Mosquito identification

Female mosquitoes were identified morphologically and scored as unfed, blood-fed or gravid. Identification of members of the *Anopheles gambiae* complex was carried out using deoxyribonuclec acid (DNA) probes on specimens collected from light traps in October 1989. DNA extracted from the tip of the abdomen of each species was amplified using a polymerase chain reaction (PASKEWITZ & COLLINS, 1989). The products of this reaction were probed with species-specific probes (supplied by Dr Frank Collins, Centers for Disease Control, Atlanta, Georgia, USA) on 0·8% agarose plates and visually inspected under ultraviolet light (PASKEWITZ & COLLINS, 1989).

Blood meal and sporozoite analysis

Mosquito bloodmeals and sporozoite positive mosquitoes were identified as described by LINDSAY *et al.* (1989b).

Bioassays

Assays were carried out to determine the insecticidal activity of permethrin on bed nets. Six randomly chosen nets were selected from each PHC village with insecticide-treated nets, 4–5 months after dipping. Mosquitoes were collected using window traps fitted to bedrooms of men sleeping under untreated nets. Using a World Health Organization (WHO) cone, 9–59 unfed *An. gambiae sensu lato* were exposed to each treated bed net, and 28–43 mosquitoes exposed to an untreated sample of polyester netting which served as a concurrent control. For all tests mosquitoes were exposed for 3 min in batches not exceeding 15 and were then transferred to paper cups and maintained on 10% sucrose. Mortality was recorded after 24 h.

Susceptibility of mosquitoes to permethrin was determined using WHO test kits with mosquitoes collected from exit traps in Sitahuma, Pakali Ba and Male Kunda. Unfed *An. gambiae s.l.* were exposed to 0·25% permethrin test paper for 10, 30, 60 and 120 min. Additional tests were carried out on mosquitoes collected from Sitahuma using 4% DDT test paper and exposures of 15, 30, 60 and 120 min.

Statistical analysis

Logarithmic transformations were applied to data when appropriate. SAS® software was used to calculate the mean logarithm of the number of vectors in each catch for each village during the 1989 rainy season. This was carried out for collections using light traps, sprays and outdoor human bait catches. Confidence intervals for the differences between the numbers of mosquitoes found in different pairs of villages were calculated as described by LINDSAY *et al.* (1993). Comparisons between years were made using data gathered during the same period in 1988 and 1989.

Human-biting rates per night for people with untreated nets were estimated by multiplying the goemetric mean number of blood-fed *An. gambiae s.l.* collected in each house in a village using spray catches by the human blood index (HBI) for each village. Seasonal inoculation rates for people with unimpregnated nets were calculated by multiplying the human-biting rates by the number of days in the season (150) and the mean seasonal sporozoite rate. Inoculation rates in the pre-intervention year were calculated as described above, assuming that the HBI in each village was the same in 1988 as in 1989.

Bioassay data collected when the control mortality exceeded 20% were excluded from subsequent data analysis.

Results

Seasonal changes in climate

The climate in the study area is typical of the sub-Sahel, with rain falling only from June to October. Temperature, relative humidity and rainfall in 1989 are shown in Fig. 1. Although saltwater enters the River Gambia in the western extremity of the study area during the dry season the salinity of water collected from the river during the middle of the wet season never rose above 3 g/L NaCl. Thus, the river water could be tol-

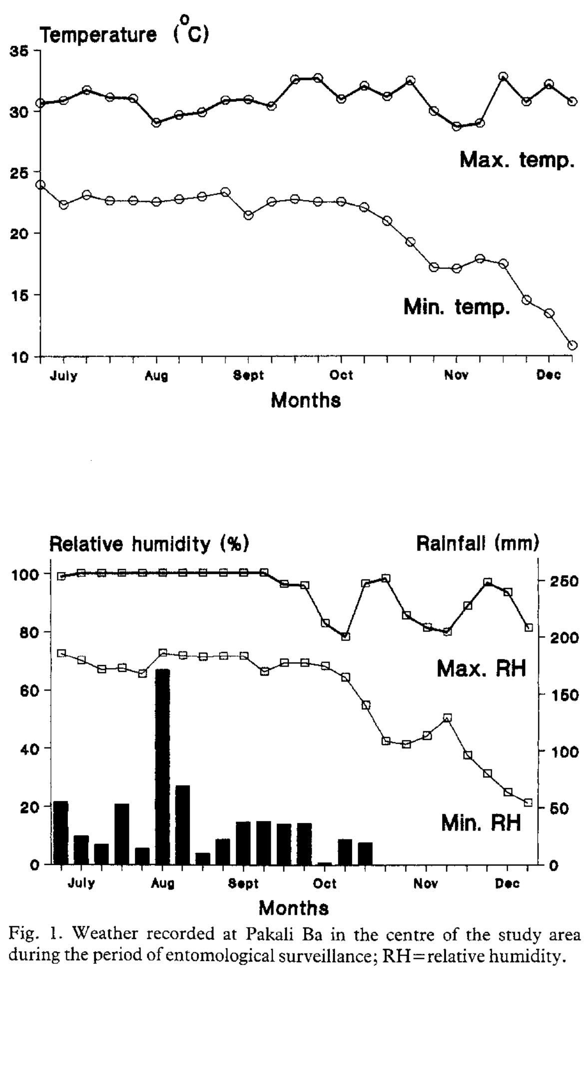

Fig. 1. Weather recorded at Pakali Ba in the centre of the study area during the period of entomological surveillance; RH=relative humidity.

Table 1. Identification of members of the *An. gambiae* complex using DNA probes

Village[a]	No. of mosquitoes sampled	*An. gambiae*	Species *An. arabiensis*	*An. melas*	Unknown
JSD	37	14 (87·5%)	0	2 (12·5%)	21
BKD	291	6 (76·2%)	4 (19·0%)	1 (4·8%)	8
KTM	100	54 (72·0%)	18 (24·0%)	3 (4·0%)	25
MKD	–	–	–	–	–
PKB	40	12 (50·0%)	12 (50·0%)	0	16
MNA	–	–	–	–	–
DSM	–	–	–	–	–
NWL	–	–	–	–	–
DGB	100	67 (77·9%)	19 (22·1%)	0	14
WGB	29	5 (38·5%)	8 (61·5%)	0	16
JBH	100	57 (100%)	0	0	43
SHM	100	28 (36·4%)	13 (16·7%)	37 (47·4%)	22
Total with treated nets	377	204 (79·1%)	49 (19·0%)	5 (1·9%)	119
Total with untreated nets	158	49 (43·8%)	25 (22·3%)	38 (33·9%)	46

[a]The first village in each pair was an intervention village; the second was a control village. Village names are given in full in the text.

erated by larvae of freshwater members of the *An. gambiae* complex.

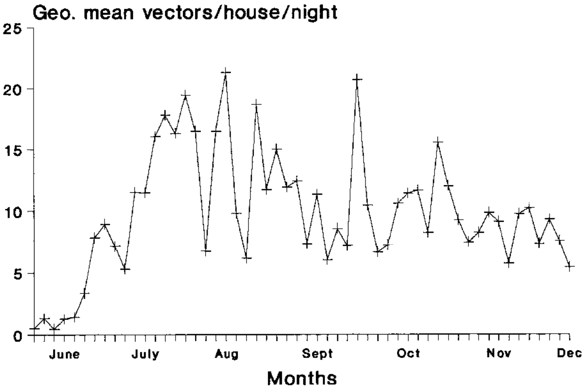

Fig. 2. Seasonal abundance of *An. gambiae s.l.* assessed from 'knock-down' collections.

Mosquito abundance

Among 68 023 mosquitoes collected using light traps during the study, 48 375 (71%) were anophelines, of which 44 887 (93%) were *An. gambiae s.l. An. gambiae sensu stricto* (68%) was the most common member of the *An. gambiae* complex found in the study area, followed by *An. arabiensis* (20%). *An. melas* occurred in a village

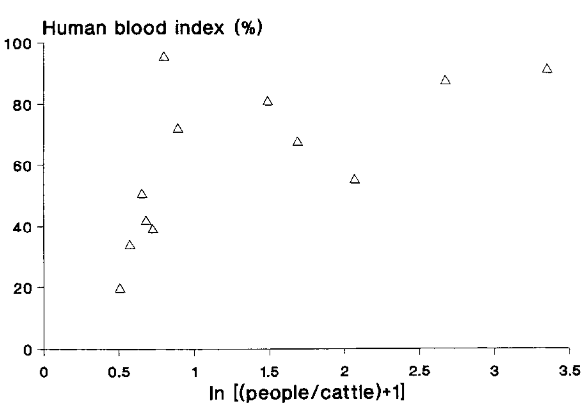

Fig. 3. Relationship between the human blood index of malaria vectors collected indoors and the relative abundance of men and cattle in each village ($\log_n$ [people/cattle] + 1).

close to the river in the western part of the study area (Table 1). Following the start of the rainy season in May the numbers of *An. gambiae s.l.* rose to a plateau and remained high until December (Fig. 2).

Generally, there was a significant inverse relationship between the numbers of *An. gambiae s.l.* collected from each village and the distance of the settlement from the river (light traps, $r=-0{\cdot}72$; $n=12$; $P=0{\cdot}01$; spray catches, $r=-0{\cdot}84$; $n=12$; $P=0{\cdot}001$; human-biting collections, $r=-0{\cdot}57$; $n=12$; $P=0{\cdot}06$). The proportion of mosquitoes which had fed on people (HBI) was inversely associated with the number of blood-fed *An. gambiae s.l.* collected using spray catches ($r=-0{\cdot}83$; $n=12$; $P<0{\cdot}001$) and directly related to the proportion of humans relative to cattle in each village ($r=0{\cdot}64$; $n=12$; $P<0{\cdot}05$) (Fig. 3).

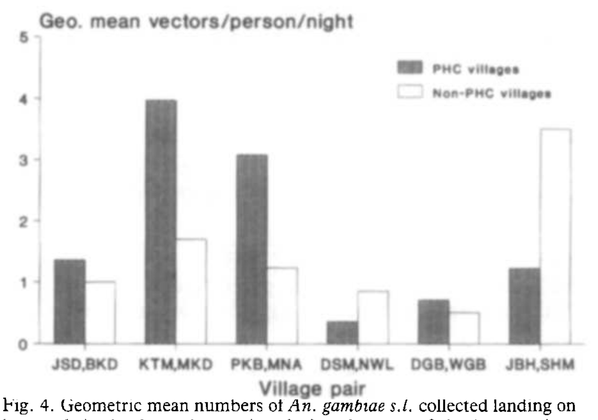

Fig. 4. Geometric mean numbers of *An. gambiae s.l.* collected landing on human baits in the early evening during the year of the interventions (1989).

Effects of permethrin-impregnated bed nets

Comparisons between mosquito numbers in villages with impregnated bed nets and those with untreated nets are shown in Figs 4–6 and summarized in Table 2. Although fewer mosquitoes were collected in PHC villages than in non-PHC villages in both years, the difference reached significance at the 5% level only for spray catches in 1989. Human-biting catches made outdoors in the early evening were similar in PHC and non-PHC villages in 1989 (6% more mosquitoes in PHC villages than in non-PHC villages).

Significantly fewer live mosquitoes were collected

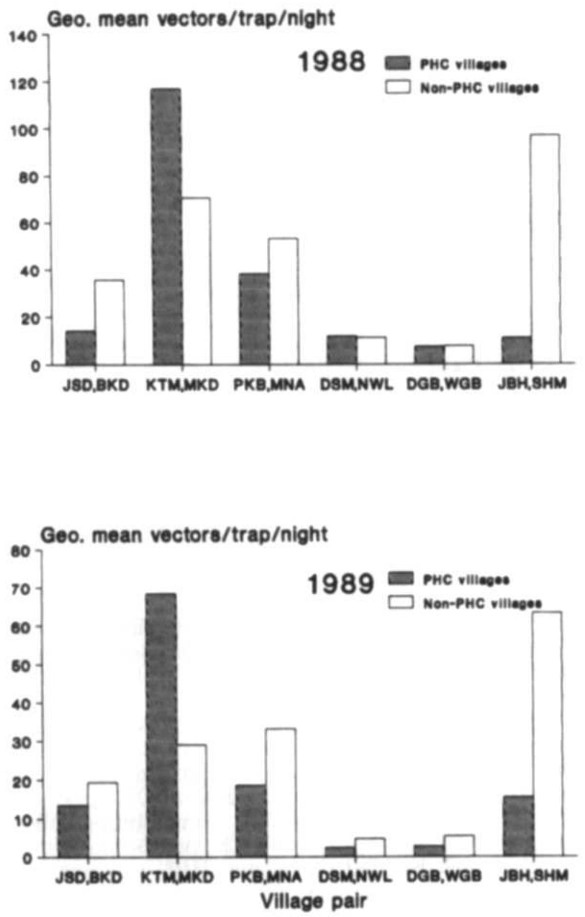

Fig. 5. Geometric mean numbers of malaria mosquitoes sampled with light traps in the study villages during the pre-intervention (upper) and intervention (lower) years (1988 and 1989).

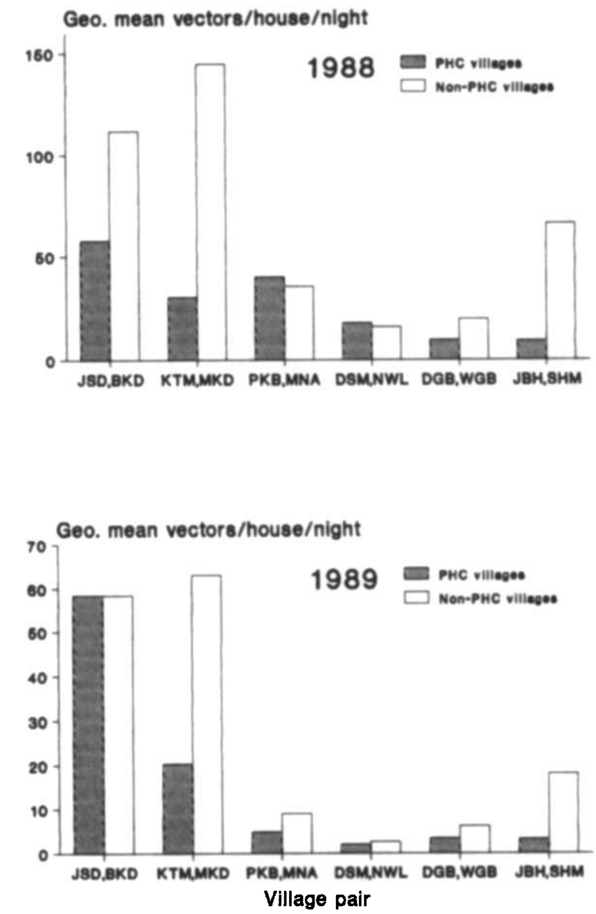

Fig. 6. Geometric mean numbers of *An. gambiae* complex mosquitoes collected with insecticide spray catches from villages during the pre-intervention and intervention years (1988 and 1989).

Table 2. Relationship between mosquito numbers in six pairs of intervention (PHC) and control (non-PHC) villages in the year before the start of a mosquito control programme in PHC villages using insecticide-treated bed nets and during the intervention year

Collection method	Pre-intervention year[a] PHC:non-PHC villages[b]	Pre-intervention year[a] Significance[c]	Intervention year PHC:non-PHC villages[b]	Intervention year Significance[c]
Light traps	0·63:1 (0·24–1·66:1)	n.s.	0·66:1 (0·32–1·40:1)	n.s.
Spray catches	0·47:1 (0·20–1·12:1)	n.s.	0·43:1 (0·25–0·73:1)	$P<0·05$
Human biting collections	– –	–	1·06:1 (0·61–1·83:1)	n.s.

[a]Data from Lindsay *et al.* (1993).
[b]95% confidence interval in parentheses.
[c]Wilcoxon matched-paired signed-ranks test; n.s.=not significant.

Table 3. Number of occasions on which live *An. gambiae s.l.* mosquitoes were found under 120 bed nets searched each week during the rainy season in primary health care (PHC) villages, in which most nets were treated, and in non-PHC villages, in which the only treated nets were those taken there by people who moved from PHC villages

	PHC Nets Treated	PHC Nets Untreated	Non-PHC Nets Treated	Non-PHC Nets Untreated
Mosquitoes				
Present	2 (0·1%)	15 (14·6%)	1 (3·8%)	765 (48·3%)
Absent	1497 (99·9%)	88 (85·4%)	25 (96·2%)	819 (51·7%)
Total	1499	103	26	1584

from under insecticide-treated bed nets compared with untreated nets, both in villages with treated nets and in those with untreated ones (Mantel-Haenszel $\chi^2=83·0$; degrees of freedom (d.f.)=1; $P<0·001$) (Table 3). Significantly fewer human-fed mosquitoes were collected from homes with untreated bed nets in villages with treated nets compared to those with untreated nets (57% reduction, $T=0$; $n=6$; $P<0·005$).

There was no significant difference between the HBI in villages with treated and untreated nets (Table 4) after adjusting for differences in levels of vector abundance ($T=6$; $n=6$; not significant [n.s.]).

Sporozoite rates were similar in villages with treated nets and those with untreated nets ($T=8$; $n=6$; n.s.) (Table 4); they were lower overall in 1989 than in 1988 before any bed nets had been treated (paired t test, $t=4·1$; d.f.=11; $P=0·002$). The drop in sporozoite rates between the pre-intervention and intervention years was similar in the intervention villages and their paired comparison villages ($T=7$; $n=6$; n.s.). Estimated inoculation rates were lower in villages with treated nets than in those with untreated ones, although this difference was

Table 4. The proportions of mosquitoes which had fed on human blood and of infective mosquitoes determined by a visual inspection of ELISA plates in 1989

Village[a]	No. of mosquitoes fed on human blood	Human blood index	No. of infective mosquitoes	Sporozoite rate
JSD	249/509	41·8%	40/4875	0·82%
BKD	339/849	33·8%	82/10255	0·80%
KTM	189/421	39·0%	40/11500	0·35%
MKD	257/1041	19·6%	47/13141	0·36%
PKB	99/105	87·6%	25/4030	0·62%
MNA	190/240	72·1%	43/5450	0·79%
DSM	41/59	67·8%	4/403	0·99%
NWL	81/85	95·3%	4/994	0·40%
DGB	39/74	50·6%	11/844	1·30%
WGB	89/110	80·9%	11/1447	0·76%
JBH	94/103	91·3%	13/2927	0·44%
SHM	292/496	55·3%	74/12324	0·60%
Total with treated nets	711/1271	55·9%	133/24579	0·54%
Total with untreated nets	1248/2821	44·2%	261/43611	0·60%

[a]The first village in each pair was an intervention village with treated nets; the second was a control village. Village names are given in full in the text.

Table 5. Estimates of sporozoite inoculation rates in primary health care (PHC villages) and in non-PHC villages before and after intervention

	Pre-intervention year (1988)		Post-intervention year (1989)	
Village[a]	Bites/ adult/ season	Infective bites/ adult/ season[b]	Bites/ adult/ season	Infective bites/ adult/ season
JSD	2380	64	1125	9
BKD	3701	110	2493	20
KTM	1076	4	672	2
MKD	2692	70	1257	5
PKB	3253	99	406	3
MNA	2376	177	693	6
DSM	1160	15	151	2
NWL	1481	0	291	1
DGB	448	80	197	3
WGB	1553	34	550	4
JBH	769	70	296	1
SHM	3366	75	960	6
Geometric mean in PHC villages	1222	20	373	3
Geometric mean in non-PHC villages	2383	33	833	5

[a]The first village in each pair was an intervention village; the second was a control village. Village names are given in full in the text.
[b]Human blood index was not determined in 1988; inoculation rates have been calculated on the assumption that it was the same as in 1989.

of borderline significance ($T=1$; $n=6$; $P=0{\cdot}06$) (Table 5).

Bioassays

Treated bed nets killed significantly more mosquitoes than untreated nets ($t=3{\cdot}7$; d.f.$=34$; $P<0{\cdot}001$). However, there was considerable variation between the killing ability of individual nets impregnated with permethrin. For example, the percentage mortality of mosquitoes exposed to treated nets which had not been washed was 42% (95% confidence interval [CI]=28%,57%). There was no significant linear decrease in the mortality of mosquitoes exposed to treated netting as the number of washes increased ($F=2{\cdot}1$; d.f.$=1$ and 54; n.s.).

Susceptibility of mosquitoes to permethrin impregnated paper is shown in Table 6. The 95% confidence intervals of probit regression lines fitted to the data suggested that the mosquito populations from the 3 villages tested were similar, although the 50% lethal exposure time (LT_{50}) calculated for Male Kunda ($LT_{50}=10{\cdot}6$ min, 95% C.I.=5·7,14·6 min) was significantly less than that for Pakali Ba ($LT_{50}=19{\cdot}3$ min, 95% C.I.=15·6,22·7 min). The LT_{50} for mosquitoes from Sitahuma was 17·7 min (95% C.I.=13·2,21·6 min) compared with 15·9 min (95% C.I.-13·6,18·1 min.) for all the villages combined. Mosquitoes collected from Sitahuma and exposed to DDT impregnated paper had an $LT_{50}=14{\cdot}5$ min (95% C.I.=11·3,16·9 min), with no mosquito surviving 60 min exposure.

Discussion

Malaria transmission in The Gambia is confined to the rainy season. On the north bank of the river appreciable numbers of vectors are found for only a few weeks each year (SNOW *et al.*, 1987; LINDSAY *et al.*, 1989a, 1989b). However, in the present study area on the south bank of the river the transmission period lasted about 5 months.

The predominant vectors in the study area were night-biting mosquitoes belonging to the *An. gambiae* complex. Of these, *An. gambiae s.s.* were more common than both *An. arabiensis* an *An. melas*, the last species being confined largely to the western fringe of the study area where the breeding sites are brackish during the early part of the rainy season. Although no major larval survey was carried out, the principal mosquito breeding sites in the area were probably the large, mainly freshwater, marshes bordering the River Gambia and its tributary, the Sofaniama Bolon, as the highest numbers of mosquitoes were found in villages nearest to the river in both 1988 (LINDSAY *et al.*, 1993) and 1989.

In contrast to the results of other workers (CURTIS *et al.*, 1990; MAGESA *et al.*, 1991) there was little to suggest that treating the majority of bed nets in a village with permethrin reduced the survival of mosquitoes. Firstly, there was no indication of a substantial decrease in mosquito numbers caught indoors in villages with treated bed nets compared to those with untreated nets. On average, PHC villages had a similar proportion of mosquitoes relative to non-PHC villages in the intervention year compared with the pre-intervention year. Using light traps, 37% fewer mosquitoes were collected in PHC villages than in non-PHC villages during the pre-intervention year, and 34% fewer during the intervention year.

Table 6. Mortality of *An. gambiae s.l.* exposed to paper impregnated with 0·25% permethrin and to untreated paper

	Village			
Exposure[a] (min)	Sitahuma	Pakali Ba	Male Kunda	Total
15	24/52 (46·1%)	22/60 (36·7%)	38/60 (63·3%)	84/172 (48·8%)
30	41/60 (68·3%)	50/68 (73·5%)	51/61 (83·6%)	142/189 (75·1%)
60	38/41 (92·7%)	58/64 (90·6%)	55/60 (91·7%)	151/165 (91·5%)
120	46/46 (100%)	64/64 (100%)	62/62 (100%)	172/172 (100%)
Untreated	2/77 (2·6%)	1/73 (1·4%)	0/65 –	3/215 (1·4%)

[a]Exposure temperature 23–32°C.

Corresponding values for insecticide spray catches were 53% and 57%. It is unlikely that in PHC villages mosquitoes deterred from entering homes with treated nets accumulated in sentinel homes with untreated nets. Studies carried out using experimental huts in The Gambia suggested that *An. gambiae s.l.* deterred from entering a hut with a permethrin-treated bed net would not be displaced to feed in adjacent huts where occupants slept under untreated nets (LINDSAY *et al.*, 1992). Secondly, the HBI of mosquitoes collected indoors in sentinel houses was similar in villages with treated bed nets and in those with untreated ones. However, this index was shown to be related to the numbers of mosquitoes in each settlement and the availability of alternative host species. Proportionately fewer mosquitoes fed on people in villages where there were large numbers of mosquitoes than in those with few vectors. This relationship may have arisen because people in villages with large numbers of mosquitoes protected themselves from biting mosquitoes better than those in villages where there were few vectors. For instance, people may go to bed earlier in villages where there are many mosquitoes. The HBI was also related to the relative availability of people and cattle in each village, with proportionately more human-fed mosquitoes occurring in settlements in which people outnumbered cattle. A similar relationship was described in Nigeria with *An. arabiensis*, although not with *An. gambiae s.s.* (WHITE & ROSEN, 1973). The readiness of Gambian *An. gambiae s.s.* to feed on species other than humans may have been due to the widespread use of bed nets which made it more difficult for a mosquito to obtain a human blood meal (LINDSAY *et al.*, 1989b). Lastly, there was no detectable reduction in sporozoite rates in mosquitoes collected from villages with permethrin-treated bed nets compared to those with untreated nets.

In the present study, the inability to demonstrate a 'mass killing' effect may have been due to the mixing of mosquito populations between villages with treated and untreated nets. A study carried out in a village nearby suggested that large numbers of mosquitoes could travel distances of 1–2 km (LINDSAY *et al.*, 1991b) and subsequent studies using mark–release–recapture methods have demonstrated substantial circulation of mosquitoes between villages in the study area (M. Thomson & M. Quinones, unpublished data). Thus, the possibility remains that the use of permethrin-treated bed nets reduced mosquito survival throughout the study area. This hypothesis is supported by the overall reduction in sporozoite rates and mosquito numbers during the intervention year compared with the previous year. However, this suggestion needs to be treated with caution since these results may have been due to the lower rainfall in 1989 than in 1988, with a consequent reduction in mosquito production.

Although at least one live *An. gambiae s.l.* was found in nearly half of all searches of untreated bed nets, they were found on only 3 occasions during 1525 searches under insecticide-treated nets. Thus, it is likely that those who slept under treated nets received far fewer mosquito bites than those who did not, despite the fact that the treatment of bed nets during this study was not optimal. Nets were treated by the villagers themselves, which resulted in a large variation of insecticide dosages between and within bed nets, and many of the nets were washed, which reduced the amount of permethrin remaining on the netting (ALONSO *et al.*, 1993b).

Results from outdoor biting collections on humans are difficult to interpret since similar collections were not made in the pre-intervention year. Whilst indoor biting rates were lower overall in PHC villages than non-PHC villages in both years, outdoor biting rates were similar in both groups of villages. As a result of the small sample size of 6 pairs of villages and the large variation in mosquito numbers between pairs, only the largest differences were significant. However, future research should consider the possibility that the use of insecticide-treated bed nets may increase the biting rates of people outdoors in the early evening. This phenomenon might occur if mosquitoes deterred from entering homes with permethrin-impregnated bed nets (LINDSAY *et al.*, 1991a; MILLER *et al.*, 1991) shifted their biting cycle from the early hours of the morning to early evening, as has been shown following DDT indoor spraying (SLOOF, 1964; TAYLOR, 1975) and following the use of permethrin-impregnated bed nets in Papua New Guinea (CHARLWOOD & GRAVES, 1987). However, no consistent effect of this kind was found in a recent study in Tanzania (MAGESA *et al.*, 1991).

Permethrin-treated nets proved extremely effective at protecting Gambian children from clinical attacks of malaria and reducing mortality (ALONSO *et al.*, 1993c). This was probably due to the action of treated nets in preventing mosquitoes entering or feeding through the nets. The outdoor biting rates obtained using men as baits may have overestimated the number of bites children received outdoors since, compared with adults, children (i) are smaller, and thus less attractive to mosquitoes, (ii) were unrestrained and could move to avoid biting mosquitoes, (iii) often slept outdoors in the early evening on raised platforms surrounded by adults, (iv) were covered frequently with a sheet or tied to their mothers' back with a cloth, or (v) went to bed earlier, particularly on nights when there were large numbers of mosquitoes.

The possible effects on malaria transmission of the administration of Maloprim® to half the children in PHC villages in the study area are uncertain. Children taking Maloprim® comprised only 10% of the total population of the villages with treated nets. However, in endemic areas, the prevalence rate of gametocytes and gametocyte density are usually highest in subjects in this age group (BRUCE-CHWATT 1951; MUIRHEAD-THOMSON, 1954; MOLINEAUX & GRAMMICIA, 1980).

Tests carried out in which mosquitoes were exposed to treated netting demonstrated a wide range of insecticidal activity between nets, which probably reflected the uneven distribution of permethrin on bed nets (ALONSO *et al.*, 1993b). Areas of netting with high dosages of insecticide may have been responsible for the finding that washing nets did not reduce killing of mosquitoes. This was a surprising result since there is overwhelming evidence to show that permethrin is washed out readily from treated netting (SNOW *et al.*, 1987; LINDSAY *et al.*, 1991c; MILLER *et al.*, 1991; NJUNWA *et al.*, 1991). However, patches of high dosages of permethrin on netting will still be potent after several washes. The patchy distribution of permethrin deposits on netting may have import ant consequences for future control programmes since it may make the emergence of resistance among vectors more likely by allowing selective survival of resistant heterozygotes. However, to date, bioassays have shown that *An. gambiae s.l.* was susceptible to permethrin in the study area. Moreover, mosquitoes were also susceptible to DDT, as assessed by bioassays, despite evidence for enhanced DDT metabolism in mosquitoes obtained from one of the study villages in 1988 (LINDSAY *et al.*, 1993).

Permethrin-treated nets provided an effective barrier against malaria mosquitoes. However, evidence for enhanced protection in villages where most people used treated nets was not found, possibly due to the circulation of mosquitoes between settlements with treated and untreated bed nets. Thus, the use of insecticide-treated bed nets throughout all villages in an area may be required before greater control can be achieved.

Acknowledgements

We express our thanks for the skill and hard work of all members of the entomology unit who worked in the South Bank Project and to Mrs M.-M. Sallah for her secretarial assistance. ICI Public Health kindly donated the permethrin. This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

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