

The impact of permethrin-impregnated bednets on malaria vectors of the Kenyan coast

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Abstract. The effects of introducing permethrin-impregnated bednets on local populations of the malaria vector mosquitoes *Anopheles funestus* and the *An.gambiae* complex was monitored during a randomized controlled trial at Kilifi on the Kenyan coast. Pyrethrum spray collections inside 762 households were conducted between May 1994 and April 1995 after the introduction of bednets in half of the study area. All-night human bait collections were performed in two zones (one control and one intervention) for two nights each month during the same period. PCR identifications of *An.gambiae sensu lato* showed that proportions of sibling species were *An.gambiae sensu stricto* > *An.merus* > *An.arabiensis*.

Indoor-resting densities of *An.gambiae s.l.* and the proportion of engorged females decreased significantly in intervention zones as compared to control zones. However, the human blood index and *Plasmodium falciparum* sporozoite rate remained unaffected. Also vector parous rates were unaltered by the intervention, implying that survival rates of malaria vectors were not affected. The human-biting density of *An.gambiae s.l.*, the predominant vector, was consistently higher in the intervention zone compared to the control zone, but showed 8% reduction compared to pre-intervention biting rates – versus 94% increase in the control zone.

Bioassay, susceptibility and high-performance liquid chromatography results all indicated that the permethrin content applied to the nets was sufficient to maintain high mortality of susceptible vectors throughout the trial. Increased rates of early outdoor-biting, as opposed to indoor-biting later during the night, were behavioural or vector composition changes associated with this intervention, which would require further monitoring during control programmes employing insecticide-treated bednets.

Key words. *Anopheles arabiensis*, *An.funestus*, *An.gambiae*, *An.merus*, malaria, permethrin-impregnated bednets, mosquito nets, Kenya Coast.

Introduction

Bednets (mosquito nets) are traditionally used to ward off mosquitoes and have been advocated as a means of personal protection against malaria vectors in Africa (W.H.O., 1986). However, torn or incorrectly tucked nets provide little additional protection and mosquitoes are adept at feeding through nets on exposed limbs (Port & Boreham, 1982). For these reasons the application of a residual insecticide (of low mammalian toxicity) to bednets was suggested in the late 1970s as a means of reinstating the effectiveness of torn or incorrectly used nets as a

man–vector barrier (Curtis *et al.*, 1990). Synthetic pyrethroids such as permethrin and deltamethrin, which have high insecticidal and excito-repellent properties, are most suitable for the treatment of bednets and have been adopted in several countries as part of national malaria control activities (Curtis *et al.*, 1990).

Malaria is the single largest cause of death among children living in tropical Africa (World Bank, 1993). Across this continent, the rates of malaria transmission and endemicity levels vary widely. The impact of insecticide-treated bednets (ITBN) upon the vector population's ability to transmit, and hence the degree of personal protection, depend largely upon the intensity of transmission in any given area. Despite encouraging effects of ITBN in reducing both morbidity and mortality among Gambian children by over 60% (Snow *et al.*, 1988; Alonso *et al.*,

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1991), those results apply to an area with low rates of sporozoite challenge with extremely seasonal vector activity (Lindsay *et al.*, 1993). The limitations of recommendations based on one transmission setting prompted the W.H.O. to replicate ITBN trials in several other areas of Africa where transmission characteristics are very different to those of The Gambia.

Accordingly, in July 1993, ITBN were introduced as part of a randomized controlled trial, conducted in coastal Kenya, to examine their role in reducing childhood mortality and severe malaria morbidity (Nevill *et al.*, 1996). This paper reports the entomological context in which the Kenyan trial was conducted and the impact of ITBNs on malaria vectors in the coastal area of Kenya.

Materials and Methods

Study area. The study area is located in Kilifi district, 60 km north of Mombasa on the Kenyan coast, extending 30 km inland and 40 km along the Indian Ocean coast north of Kilifi town. The area was designated in 1989 for intensive entomological (Mbogo *et al.*, 1993b, 1995), demographic (Snow *et al.*, 1994) and epidemiologic studies (Snow *et al.*, 1993) of malaria. The principal vectors of malaria are the *Anopheles gambiae* Giles complex with a minor role played by *Anopheles funestus* Giles. These two vectors yield on average ten sporozoite inoculations per person per year. Among the people inhabiting this geographical area, annual rates of *Pfalciparum* challenge range from less than one to sixty per person (Mbogo *et al.*, 1995). Despite these annual rates of *Pfalciparum* inoculation being lower than in most parts of tropical Africa, it has been estimated that at least one in fifteen children will develop severe life-threatening malaria before their fifth birthday (Snow *et al.*, 1993).

The study population comprises approximately 60,000 inhabitants living mainly in traditional style houses (walls of sticks and mud) with a coconut thatch roof. Unscreened windows, holes in the walls and large open eaves provide easy access for mosquitoes. Homesteads are scattered and separated from one another by open farmland. Maize is the staple crop cultivated for home consumption; cashews and coconuts are grown as cash crops. During the 1989 national Kenyan census the study area was divided into seventy-two enumeration zones, of which thirty-eight were randomly allocated to receive ITBN.

Green polyester 100 denier mosquito nets (SiamDutch, Thailand) were issued to be used over all beds within the intervention zones and impregnated with 25% permethrin (*cis:trans* 40:60) emulsifiable concentrate (Imperator, ICI, U.K.) to achieve a target dose of 0.5 g of permethrin per m² of netting. Nets were re-impregnated every 6 months to coincide with the two main rainy seasons: in May, the beginning of the long rains; and October, towards onset of the short rains. In the intervention area, people were asked not to wash their nets until immediately before the next re-impregnation. The remaining thirty-four zones served as the contemporaneous non-intervention control area where bednet ownership was less than 6% (Snow *et al.*, 1992).

The study area was mapped using a hand-held satellite navigational system (Trimble Navigation Europe, U.K.) and computerized using MapInfo^R software (Troy Ltd, U.S.A.).

Entomological surveillance. One homestead from each zone was randomly sampled for mosquitoes by Pyrethrum spray-catch (PSC) each month (May 1994 to April 1995); no homestead was sampled more than once. Nine zones (five intervention and four control) were excluded from the sampling frame because they formed part of ongoing entomological studies since 1989. Houses were visited in the morning (07.00–11.30 hours) and occupants asked to tie their bednets up away from the bed. White sheets were laid on the floors and the rooms sprayed with pyrethrum aerosol. All mosquitoes knocked down were collected into labelled petri dishes lined with moist cotton wool and taken to the laboratory at Kilifi for further investigation.

Pre-intervention all-night catches of human-biting mosquitoes were undertaken once a week at four sentinel households per zone (five intervention and four control), between May 1992 and April 1993. Post-intervention human-bait collections were performed at one control and one intervention zone (drawn from the nine pre-intervention zones and excluded from PSC catches) for two nights each month between May 1994 and April 1995. Pairs of experienced catchers recruited from the study area were positioned either indoors or outdoors at each site and collections made from 18.00 until 07.00 hours. Catchers rotated in shifts and used aspirators and torches to catch mosquitoes which landed on exposed limbs. Each hourly catch was placed into a pre-labelled polystyrene container and taken to the laboratory at Kilifi for assessment.

Laboratory procedures. Mosquito species were identified morphologically and scored as unfed, blood-fed or gravid. A proportion of *An.gambiae* s.l. females collected by both PSC and all-night biting catches were identified to sibling species by the method of polymerase chain reaction, PCR (Paskewitz & Collins, 1990). Primers used were specific for *An.gambiae* s.s., *An.arabiensis* and *An.merus*, members of the *An.gambiae* complex found at the Kenyan coast (Mosha & Petrarca, 1983). Samples of *An.gambiae* s.l. collected on human bait were dissected for parity determination as described by Detinova (1962). Mosquitoes collected by PSC were prepared for sporozoite enzyme-linked immunosorbent assay (ELISA) testing using monoclonal antibodies to detect circumsporozoite proteins of *Pfalciparum* (Wirtz *et al.*, 1987). Tests were assessed visually for positivity (Beier & Koros, 1991). Bloodmeals were identified by direct ELISA using anti-host (IgG) conjugates against human, cow and goat (Beier *et al.*, 1988).

Bioassay, bioavailability and susceptibility tests. Nets were randomly selected from intervention households, between 1 and 17 months after they were issued. These nets were visually inspected and coded as either clean or dirty, and for the number of re-impregnations each net had received. Bioassay cones (W.H.O., 1975) were attached to the nets by means of elastic bands whilst the nets were hung upright in the laboratory. Two cones were used, one placed at the top of the net and the other on the lower portion toward the floor. Twenty laboratory-colonized female *An.gambiae* s.s. were introduced to each cone and exposed to the netting for 3 min before they were removed to paper cups. Delayed mortality was recorded after the mosquitoes had been left in the paper cups for 24 h with adequate sugar water in an ambient temperature of 25°C and a relative humidity of 72%. Four repeats per net were performed. Identical procedures were followed for untreated nets to serve as controls. Mortality

was corrected for control mortality where the latter exceeded 20% of exposed mosquitoes.

Sample swatches of netting fabric were collected for high-performance liquid chromatography (HPLC) immediately after nets were impregnated for the first time and 11 months later, after two re-impregnations. HPLC assays were conducted at the Centers for Disease Control, Atlanta, U.S.A., to determine the concentration of the active *cis* isomer of permethrin per m² of netting.

Susceptibility of wild-caught female mosquitoes, collected from an area adjacent to the study area, was determined in February 1995 using the W.H.O. (1981) test kit and procedure. Unfed *An.gambiae s.l.* females ($n = 415$) were exposed to 0.25% permethrin test paper for 1 h. Delayed mortality was measured 24 h post-exposure to the permethrin or control papers, and corrected if control mortality exceeded 20%.

Statistical analysis. The mean number of mosquitoes per house was calculated (from PSC data) for each of the zones sampled over the 12 months of surveillance (Table 1). The annual means of the thirty-three intervention zones were compared with the annual means of the thirty control zones using a Mann-Whitney U test (given their non-normal distribution). Human blood indices, sporozoite rates, parity and man-biting rates were analysed post-intervention using a Chi-square test, or controlling for pre-intervention rates using a Mantel Haenzel Chi-square test.

Results

A total of 762 houses were sampled by PSC between May 1994 and April 1995. Of the 362 houses sampled within the non-intervention (control) area, 31.5% (114) yielded at least one *An.gambiae s.l.* or *An.funestus*, compared to only 11.3% (45/

400) of the intervention houses sampled during the same period ($\chi^2 = 45.9$, $v = 1$, $P < 0.001$). As described previously (Mbogo *et al.*, 1995), large between-zone variation in vector abundance occurs within this relatively small geographical area (Table 1). Comparing the ranks of the mean zonal densities of either *An.gambiae s.l.* or *An.funestus* per house indicates, before intervention, a significant difference of indoor-resting mosquito densities between intervention and non-intervention areas (Mann-Whitney U test, $P < 0.0001$). Overall, post-intervention, there was a nine-fold reduction of the indoor-resting densities of both *An.gambiae s.l.* and *An.funestus* associated with ITBN use. Fig. 1 shows that the typical peaks of *An.gambiae s.l.* density during the long rains (May–August) and the short rains (November–December) were virtually eliminated in areas where ITBN were used.

Composition of the *An.gambiae* complex differed significantly between non-intervention (control) and ITBN intervention areas (Table 2). Proportions of *An.arabiensis*, *An.gambiae s.s.* and *An.merus* were 7%, 49% and 44% respectively among seventy-two specimens identified by PCR from the intervention area, compared with 11%, 83% and 6% of these three sibling species, respectively, among 165 specimens identified from the non-intervention area. PSC densities of all three species were significantly different both between treatment areas and between species within areas (Table 2). Per house sampled, the intervention area had 2.9-fold more *An.merus* but 4-fold less *An.arabiensis* and 4.3-fold less *An.gambiae s.s.* than the non-intervention (control) area.

In houses with impregnated bednets, significantly fewer *An.gambiae s.l.* were found to be blood-fed and their human-blood index was lower than in control houses, although this difference was not statistically significant (Table 3). There were no significant differences between areas in the proportion of *An.gambiae s.l.* with detectable *P.falciparum* CS protein and

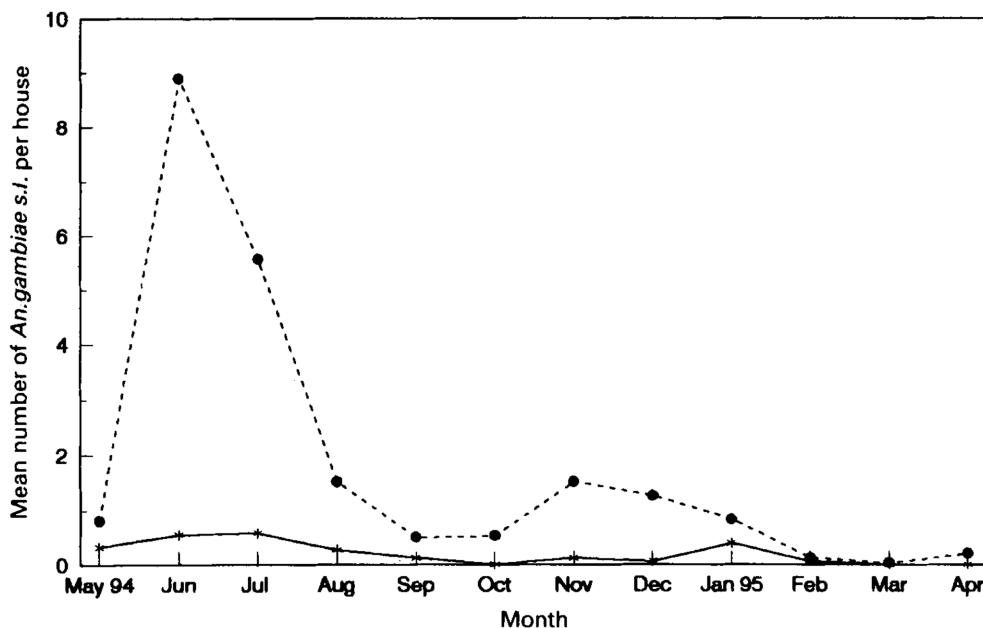


Fig. 1. Monthly abundance (May 1994 to April 1995) of indoor-resting *An.gambiae s.l.* females among households where ITBN were used (closed line) and households in a non-intervention (control) zone without bednets (dotted line).

Table 1. Numbers of houses sampled by pyrethrum spray collection: *An.gambiae* s.l. and *An.funestus* collections in each enumeration zone Intervention and control areas.

Intervention (ITBN)						Non-intervention (control)					
Zone	A No. houses	B No. <i>An.gambiae</i> s.l.	C No. <i>An.funestus</i>	Anopheles density (B + C)/A	Zone	A No. houses	B No. <i>An.gambiae</i> s.l.	C No. <i>An.funestus</i>	Anopheles density (B + C)/A		
01	12	0	0	0.00	02	12	33	1	2.83		
03	12	16	0	1.33	04	12	11	1	1.00		
09	12	2	0	0.17	05	12	29	0	2.42		
10	13	2	0	0.17	06	12	8	0	0.67		
11	12	0	0	0.00	07	12	13	0	1.08		
12	12	1	0	0.08	08	12	10	0	0.83		
13	12	2	0	0.17	16	12	1	0	0.08		
14	12	1	0	0.08	17	12	3	0	0.25		
18	12	2	1	0.25	20	12	4	0	0.33		
21	12	0	0	0.00	23	12	4	1	0.42		
22	12	2	0	0.17	27	12	4	0	0.33		
24	12	2	0	0.17	34	12	1	0	0.08		
25	12	0	1	0.08	36	12	4	3	0.58		
26	12	1	0	0.08	37	12	3	0	0.25		
28	12	1	0	0.08	43	12	19	1	1.67		
30	12	5	0	0.42	44	12	60	10	5.83		
31	12	2	0	0.17	45	12	11	5	1.33		
32	12	3	0	0.25	46	12	63	4	1.46		
38	12	6	0	0.50	52	12	9	0	0.75		
47	12	0	1	0.08	53	12	16	3	1.58		
48	12	1	0	0.08	56	12	15	1	1.33		
49	12	0	0	0.00	57	12	83	33	9.67		
50	12	1	0	0.08	58	12	43	6	4.08		
54	12	5	0	0.42	59	12	71	4	6.25		
60	12	2	0	0.17	65	12	6	0	0.50		
61	12	6	0	0.50	66	12	23	0	1.92		
62	12	1	0	0.08	75	12	13	1	1.17		
63	12	0	0	0.00	78	12	37	5	3.50		
70	12	13	1	1.17	80	13	26	2	2.15		
71	13	2	0	0.15	81	13	24	2	2.17		
76	12	0	0	0.00							
77	13	1	0	0.08							
79	13	2	0	0.15							
Total	400	82	4	0.22			362	83	2.02*		

* Weighted mean density of *Anopheles* per house.

Table 2. Numbers (%) of each sibling species of the *Anopheles gambiae* complex collected in each area by pyrethrum spray collections indoors.

	Area		Difference		χ^2	<i>P</i>
	Non-intervention (control)	Intervention (ITBN)				
No. houses sampled by PSC	362	400				
Species						
<i>arabiensis</i>	18 (11)	5 (7)	7	<0.01	23	$\chi^2 = 167$, $v = 2$, $P < 0.0001$
<i>gambiae</i>	137 (83)	35 (49)	60	<0.0001	172	
<i>merus</i>	10 (6)	32 (44)	12	<0.001	42	
Total <i>An.gambiae</i> s.l.	165 (100)	72 (100)	48	<0.0001		

the proportion of parous female *An.gambiae* s.l. collected on human bait (Table 3).

Table 3 also gives the mean numbers of *An.gambiae* s.l. females/person/night landing on human bait, both indoors and outdoors, during periods of 12 months before and after intervention in both the ITBN area (185 man-nights pre- and 64 man-nights post-intervention) and the non-intervention area (182 man-nights pre- and 70 man-nights post-intervention), sampling consistently from the same two houses in each area. After introduction of ITBN, greater proportion of *An.gambiae* s.l. were caught biting outdoors (30.3%) in the intervention area, compared to the non-intervention area (23.2%): Mantel Haenzel allowing

for differences pre-intervention: $\chi^2 = 26.0$, $P < 0.0001$. Fig. 2 suggests that there was a tendency toward earlier biting activity inside houses where ITBN were in use compared to the biting cycle in control houses: 12% of bites occurred before 22.00 hours in houses with ITBN, compared to only 7% in control houses.

Calculating the product of the monthly man-biting rates and sporozoite rates shown in Table 3 (determined from all-night human bait catches in only two zones) suggests that the average annual sporozoite inoculation rate per person was not significantly reduced by the use of ITBN.

In an attempt to examine the possible influence on mosquitoes

Table 3. *An.gambiae* s.l. collections from control (non-intervention) and ITBN (intervention) areas, pre-intervention (May 1992 to April 1993) and post-intervention (May 1994 to April 1995). Proportions bloodfed, parous and sporozoite positive from PSC samples; man-biting rates and sporozoite inoculation rates from human bait catches.

	Non-intervention (control) (n = 362)	Intervention (ITBN) (n = 400)	Difference	
			χ^2	<i>P</i>
PSC surveys				
Blood-fed (%)	55.5% (359/647)	28.0% (23/82)	20.9	<0.001
Human Blood Index (%)	86.4% (242/280)	80.0% (16/20)	0.09	>0.75
Sporozoite rate (%) (csp positive)	5.0% (32/647)	4.9% (4/82)	0.01	>0.9
Human-bait surveys				
Parity (%)				
Before	65.5% (129/197)	63.2% (427/676)	0.11	<0.75
After	63.4 (78/123)	54.1% (242/447)	1.50	<0.25
Difference	$\chi^2 = 0.05$ <i>P</i> < 0.75	$\chi^2 = 3.60$ <i>P</i> < 0.05		
Man-biting rate* per man per night (n)				
Indoors				
Before	5.3 (182)	93.4 (185)		
After	3.7 (70)	23.5 (64)		
Difference	-1.6	-69.9		
Outdoors				
Before	0.15 (182)	1.14 (185)		
After	0.94 (70)	10.20 (64)		
Difference	+0.79	+9.06		
Annual sporozoite inoculation rate†				
Before	18.0	59.6		
After	35.0	54.1		
Difference	+94%	-9.2%		

* Sampling done in the same rooms before and after intervention, two houses per zone (*n* = number of man-nights collection).

† Calculated as a sum of the products of the monthly indoor man-biting rates and monthly sporozoite rates.

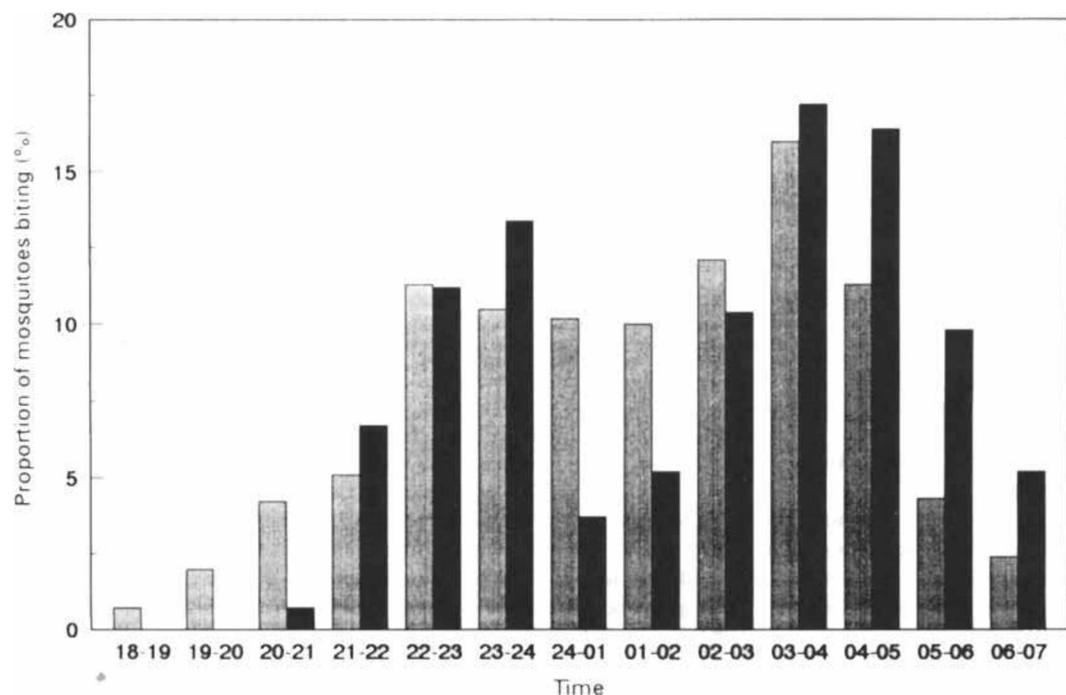


Fig. 2. Biting cycle (hourly percentage on human bait) of *An.gambiae* s.l. in houses where ITBN were used (light bars) and in a non-intervention zone where bednets were not used (dark bars).

(deleterious or beneficial effects) entering houses in the control (non-intervention) area close to the intervention area, we used longitude and latitude to establish precise distances from intervention zones of thirty-five houses in control zones 57, 58 and 59, selected because they generated the largest numbers of mosquitoes. Distances were classified as more or less than 400 m from the intervention area. Among eleven households within 400 m, 9% had at least one mosquito, significantly less than 46% of twenty-four households at a distance of more than 400 m from the intervention area. ($\chi^2 = 4.5$, $v = 1$, $P < 0.05$).

Details of user compliance of the intervention will be presented elsewhere (Some *et al.*, in prep.). Potency of the permethrin applied to bednets remained high throughout the trial. HPLC analyses of the nets indicated that, following the first treatment, the average concentration of cis-permethrin was 0.43 g/m² (95% CL 0.34–0.52) on thirty-six nets. Eleven months later, following re-impregnation after 6 months, the average concentration on thirty-six nets was 1.36 g/m² (95% CL 1.21–1.51).

Table 4. *An.gambiae* s.l. mortality within 24 h following exposure for 3 min to permethrin-impregnated bednets retrieved from intervention villages in May 1994 and March 1995.

Months in use (no. of impregnations)	Percentage mortality (no. of nets tested)
0 (1)	80.8 (4)
4–8 (2)	78.3 (13)
10 (3)	96.0 (9)
17 (4)	99.8 (5)

Permethrin susceptibility tests of local wild-caught *An.gambiae* s.l., using the diagnostic dosage of 1 h exposure to 0.25% permethrin in W.H.O. (1975) test kits, gave a mortality-rate of 94.5% when tests were undertaken 21 months after the trial began.

Bioassay tests with *An.gambiae* s.l. (3 min exposure, 24 h mortality) on various nets that had been used for up to 8 months following their initial impregnation in July 1993 gave greater than 78% kill (Table 4). Nets re-impregnated in April and November 1994 showed an increased killing capacity of 96–99.8%. Interestingly, nets which were found to be dirty with cooking soot had higher killing effects (94.1%) than nets which were clean (84.5%).

Discussion

Our results demonstrate that permethrin-impregnated bednets exert a major impact upon the indoor-resting abundance of the principal vectors of *P.falciparum* malaria in coastal villages of Kenya. Indoor-resting densities of *An.gambiae* s.l. and *An.funestus* were 9 times lower in houses where ITBN were in use, compared to households where no nets were used. This had the additional effect of eliminating the typical seasonal peaks in vector density usually seen in this part of Kenya (Fig. 1), despite evidence that more of the vector species were biting outdoors (Table 3). These findings are consistent with other studies of synthetic pyrethroid treated bednets or curtains in Africa (Lines *et al.*, 1987; Majori *et al.*, 1987; Lindsay *et al.*, 1989, 1993; Magesa *et al.*, 1991; Robert & Carnevale, 1991; Beach *et al.*, 1993). The precise effect in each of these areas is difficult

to compare, given the inherent differences in the sampling procedures used within each study. We opted not to use light traps (Lines *et al.*, 1991; Mbogo *et al.*, 1993a) in our estimation of vector abundance, because they tend to be less efficient in areas of low vector abundance (such as our study area) and have been shown to over-estimate parity rates in this area of Kenya (Petrarca *et al.*, 1991). Furthermore, we required a simple and rapid means of monitoring endophilic mosquitoes over a wide geographical area, so as to truly reflect the impact of ITBN within our entire study population. Intensive entomological surveillance limited to a few sites – as suggested by the W.H.O. (1991) – can yield unrepresentative results in areas where marked over-dispersion of vectors is common. However, it could be argued that reductions of indoor-resting densities – as determined by PSC – may simply reflect increased excito-repellency of the insecticides and not a reduction in the numbers of vectors coming to feed. Indeed, studies with exit traps in The Gambia have shown an increased rate of exophily due to ITBNs indoors (Snow *et al.*, 1987; Miller *et al.*, 1991). In addition, however, there is clear evidence that houses with pyrethroid-treated fabrics tend to significantly deter entry of vectors into the house (Lines *et al.*, 1987; Lindsay *et al.*, 1991). Further evidence from our study that man–vector contact was reduced is shown by the very highly significantly reduced proportion of *An.gambiae s.l.* found blood-fed in the early-morning PSC samples (Table 3). Human bait catches, however, revealed no significant reduction in the number of sporozoite inoculations an unprotected individual is likely to receive per year when living in a household where ITBN were used, compared to living in a house where no nets were in use. Whereas the sporozoite inoculation rate increased by 94% in the non-intervention area, for unaccountable (probably climatic) reasons between pre- and post-intervention years, it decreased by 8.3% in the ITBN intervention area, a significant reduction.

Interestingly, our study did not demonstrate a significant reduction in the actual sporozoite rate or parity (an index of longevity) among vectors sampled from ITBN intervention zones compared to non-intervention (control) zones. Similar results were obtained in the Gambia, where bednets were also impregnated with permethrin 0.5 g/m², and this has been interpreted as a probable lack of any so called ‘mass effect’ upon the vector population (Lindsay *et al.*, 1993; Thomson *et al.*, 1995). Mass effects would be difficult to prove in most field study designs, because the intervention could affect mosquito abundance in the untreated (control) as well as treated (ITBN) areas, as shown by the overall reductions compared to pre-intervention data in both The Gambia (Lindsay *et al.*, 1993) and Burkina Faso (Robert & Carnevale, 1991). Hence Lines *et al.* (1987) and Lindsay *et al.* (1991) argued that, although individuals appear to be protected by ITBN against the bites of vector mosquitoes, there is no evidence that this increases the biting rate on unprotected neighbours. Under fortuitous circumstances, there may be some reduction of biting on people without ITBN if they are sufficiently closely associated with ITBN users to be afforded some protection. We have tried to assess this ‘community protection’ by studying three control communities in close proximity to intervention communities, comparing vector abundance by distance from the nearest houses where ITBN were widely employed. This analysis indicated that, within the non-intervention area, fewer houses closest to the intervention area had any malaria

vectors compared to those further away.

The dipping procedures used for bednet impregnation during this trial provided adequate target treatment concentration of 0.5 g/m² (over 76% of all netting samples tested had excess of this figure), giving bioassay mortalities in excess of 80% throughout the study, increasing to almost 100% following multiple re-impregnations at half-yearly intervals (Table 4).

Perhaps the greatest concern raised by this study is the observation that a significant proportion of malaria vectors appeared to bite earlier in the evening in houses where ITBN were used, with a greater tendency toward exophagy rather than the typical endophagy of most anthropophilic *An.gambiae s.l.* Furthermore, there was an apparent shift in sibling species composition of the *An.gambiae* complex following the introduction of ITBN. Both *An.merus* and *An.arabiensis* have slightly different biting cycles to *An.gambiae s.s.* (Iyengar, 1962; White, 1974; Mosha & Petrarca, 1983). Earlier biting is associated with use of permethrin-treated bednets in Papua New Guinea (Charlwood & Graves, 1987). As the biting cycle change occurred immediately after installation of ITBNs in our study, in conjunction with the lack of evidence for a mass-killing effect, we conclude that the earlier biting reflects either an immediate intraspecific behavioural effect or a change in vector species proportions within the *An.gambiae* complex, and was not the result of selection for evolved behavioural resistance. Among our Kenyan study population, people usually ‘go to bed’ at 21.00–22.00 hours (unpublished data) and most children retire earlier, so their customs limit the opportunities for vectors to bite them, especially when they sleep under bednets. If ITBN are increasingly to be employed against malaria in tropical Africa, their effects on mosquito behaviour and insecticide susceptibility (cf. Vulule *et al.*, 1996) should be monitored.

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