Medical and Veterinary Entomology (1998) **12**, 52–59

Permethrin-impregnated bednet effects on resting and feeding behaviour of lymphatic filariasis vector mosquitoes in Kenya

CLAUS BØGH, ERLING M. PEDERSEN, DUNSTAN A. MUKOKO* and JOHN H. OUMA*

The Danish Bilharziasis Laboratory, Charlottenlund, Denmark and *Division of Vector Borne Diseases, Ministry of Health, Nairobi, Kenya

Abstract. The impact of permethrin-impregnated bednets on resting and feeding behaviour of mosquito vectors of *Wuchereria bancrofti*, causing human lymphatic filariasis was studied in six pairs of villages (treated and untreated) before and after intervention. The study villages were in Kwale District, near the coast of Kenya, where Bancroftian filariasis is highly endemic, transmitted by a combination of both anopheline and culicine mosquito vectors. Mosquitoes were collected weekly in each village, indoors (using pyrethrum spray catches) and outdoors (using pit traps) during 3–4 months following the long rainy season. Of the filariasis vector species of mosquitoes collected in 1994 before intervention, 33.6% were members of the *Anopheles gambiae* complex, 30% were *An. funestus* and 36.4% were *Culex quinquefasciatus*. PCR analysis of the *An. gambiae* complex species collected in 1995 demonstrated that 98.5% were *An. gambiae sensu stricto*, 1% *An. arabiensis* and 0.5% *An. merus*.

Introduction of impregnated bednets in 1995 significantly reduced the number of indoor-resting *An. gambiae s.l.* by 94.6% and *An. funestus* by 96.7%, but there was no change in the number of *Cx quinquefasciatus* collected indoors. The number of outdoor-resting *An. gambiae s.l.* was significantly reduced, whereas densities of *An. funestus* and *Cx quinquefasciatus* remained unaffected outdoors. ELISA analysis of mosquito bloodmeals showed a shift from human to animal feeding after the introduction of treated nets. The human blood index (HBI) for indoor resting *Cx quinquefasciatus* was reduced from 93.1% to 14.4%. Vector potential based on the HBI and mosquito density was estimated to be reduced by 99% for *An. gambiae s.l.*, 98% for *An. funestus* and 97% for *Cx quinquefasciatus* and vectorial capacity would be suppressed even more by the impact on the vector survival rates (not measured). These results suggest that permethrin-impregnated bednets give effective personal protection against transmission of *W. bancrofti* by *An. gambiae, An. funestus and Cx quinquefasciatus* in East Africa.

Key words. *Anopheles funestus, Anopheles gambiae, Culex quinquefasciatus,* permethrin-impregnated bednets, lymphatic filariasis, filariasis vectors, mosquito resting and feeding behaviour, vector control, Kenya.

Introduction

Pyrethroid-impregnated bednets are being used increasingly for personal protection against malaria in the tropics, effectively reducing the incidence of malaria infection (Choi *et al*., 1995) and morbidity and mortality from this disease (Alonso *et al*., 1993; Binka *et al*., 1996; Nevill *et al*., 1996). Permethrin-impregnated nets deter some mosquitoes from entering houses and they kill or irritate those mosquitoes which actually contact the nets (Rozendaal, 1989; Pleass *et al*., 1993). The effects depend largely on the dose and type of pyrethroid, the type of netting and the species of mosquito (Hossain *et al*., 1989; Rozendaal, 1989; Wu Neng *et al*., 1991; Curtis *et al*., 1996).

Correspondence: Erling M. Pedersen, The Danish Bilharziasis Laboratory, Jaegersborg allé 1D, 2920 Charlottenlund, Denmark. Fax: +45 39 62 61 21. E-mail: dbl@bilharziasis.dk

© 1998 Blackwell Science Ltd

The effect of use of impregnated bednets on malaria vectors has been studied extensively at community levels in many parts of the tropics (Rozendaal, 1989; Curtis *et al*., 1990). In Tanzania, East Africa, permethrin-impregnated bednets reduced the malaria inoculation rate by 90% (Magesa *et al*.,1991) and in Guinea Bissau, West Africa, Jaenson *et al*. (1994) achieved malaria control with a 95% reduction in the number of indoor-resting *Anopheles gambiae* Giles complex mosquitoes. In The Gambia, however, no reduction was achieved in the human blood index (HBI) of indoor-resting *An. gambiae* (Lindsay *et al*., 1993), nor of outdoor-resting *An. gambiae* in Tanzania (Magesa *et al*., 1991).

One would expect impregnated bednets to be more effective against the transmission of lymphatic filariasis than malaria, as more infective bites are required to produce a patent infection of lymphatic filariasis (Southgate, 1992). An intervention trial was carried out, therefore, to measure the effect of permethrin-impregnated nets on the transmission of *Wuchereria bancrofti* (Cobbold) on the Kenya coast. The impact on filariasis transmission and infection status will be described elsewhere (Mukoko *et al*., in prep.). The aim of this study was to investigate possible effects on the resting and feeding behaviour of filariasis vectors in East Africa: *Anopheles gambiae* Giles *sensu lato*, *Anopheles funestus* Giles *sensu stricto* and *Culex quinquefasciatus* Say, formerly known as *Cx fatigans* Wiedemann (White, 1971), after permethrin-impregnated bednets were introduced for use by entire communities. It was of particular interest to determine the effect of these nets on *Cx quinquefasciatus*, as this species is more tolerant than *Anopheles* mosquitoes to permethrin (Hossain *et al*., 1989; Wu Neng *et al*., 1991; Mutinga *et al*., 1992; Curtis *et al*., 1996). In a Tanzanian village, bednets impregnated with permethrin 200 mg/m^2 gave little reduction of indoor resting *Cx quinquefasciatus* (Magesa *et al*., 1991), so it was argued that permethrin-impregnated bednets would not be effective against the transmission of *W. bancrofti* by *Cx quinquefasciatus*.

Materials and Methods

The study area comprised six pairs of villages in Kwale district, south of Mombasa, in the Coast Province, Kenya. The area is characterized by two rainy seasons, April–June and October–November, with rainfall averaging 1250 mm annually. Two rivers, the Umba and Ramisi, and smaller permanent and seasonal streams drain the area. Seasonal swamps are also present and some are used for rice cultivation during the rainy season. Goats and chickens are kept by many householders, with cattle in some of the villages. The area is endemic for lymphatic filariasis transmitted by all three vector species (Wijers, 1977).

Representative villages, at least 1 km apart in the southern division of Kwale District, were paired on the basis of geographical position and surrounding habitats. One village of each pair was selected randomly for intervention. The intervention and control villages in each pair, respectively, were: (1) Shirazi and Gazi, (2) Mwendowabure and Bodo, (3) Zigira and Magaoni, (4) Mwachande and Vwivwini, (5) Mwena and Makwenyeni and (6) Kidomaya and Tsuini. Village populations ranged from 375 to 1151 inhabitants. During June and July 1995, the intervention villages were supplied with enough permethrin-treated bednets to allow all the inhabitants to sleep under treated nets. One person from each house was trained in use of the nets. Soon after the nets were distributed, all households were visited to ensure that the nets were correctly installed. Subsequently, early morning visits were made randomly to several houses in each village to check that the nets were used properly. The nets supplied were rectangular, made of polyester (100 denier, mesh 156, Siamdutch, Bangkok) and impregnated with 500 mg/m^2 permethrin (Peripel, AgrEvo) and they were re-impregnated every six months.

Entomological surveillance

Mosquito surveys were undertaken weekly, indoors and outdoors, following the long rainy season during the pre-intervention year (June–August 1994) and from late July to October in 1995, starting just after the introduction of the bednets. Indoor-resting mosquitoes were collected weekly from two houses in each village: one in the centre and another at the periphery of the village. Pyrethrum spray catches (PSC) of mosquitoes in the bedroom of each house were performed at 0700–1000 hours, as described by Service (1976), using 1% pyrethrum and 1.7% piperonyl butoxide dissolved in kerosene. In villages with relatively few mosquitoes, PSCs were undertaken in additional houses to supplement the number of specimens collected for bloodmeal analysis. Two pit traps were constructed in each village, when possible in a shaded place, within 10 m of each primary PSC house. The pit traps were 1.2 m square and 1.5 m deep, covered with a raised thatched roof allowing for mosquito entrance. Two chambers 30 cm deep were dug in each side of the pit trap, 1 m from the bottom (Service, 1976). For weekly sampling of outdoor-resting mosquitoes, each pit was searched for 15 min, always by the same person using torch-light and aspirator, during the same time that the spray-catches were performed. In Bodo it was impossible to make pit traps because the water table was only 60 cm below ground level. Instead, two 180 litre oil drums were used, laid horizontally with one end open, heaped with soil and coconut leaves. Collected mosquitoes were brought to the laboratory in small containers in a cooling box.

Mosquito identification

In the laboratory mosquitoes were killed by freezing and stored at –20°C, then sorted and identified to species on the basis of morphological characters (Edwards, 1941; Gillies & DeMeillon, 1968). Bloodfed females (up to 1/3 gravid) were used for bloodmeal analysis. In 1995, samples of *An. gambiae* complex mosquitoes were collected during August–September, at the end of the rainy season, from houses in each of the twelve villages and tested for sibling species identification. Specimens were stored in a container with silica gel until polymerase chain reaction (PCR) analysis using primers for

three members of the *An. gambiae* complex (Scott *et al*., 1993) known to occur at the Kenyan coast: *An. gambiae* Giles *sensu stricto*, *An. arabiensis* Patton and *An. merus* Dönitz (Mosha & Petrarca, 1983).

Bloodmeal identification

Blood-filled abdomens of recently fed mosquitoes were squashed individually on filter paper (Schleiser & Schuell No. 2992) (Evengård *et al*., 1988) by application of pressure from a finger tip covered with disposable plastic tape. The plastic tape was changed and the knife for cutting the abdomen was cleaned before handling another specimen. Bloodmeals on filter papers were dried and stored in a desiccator at 4°C. Each bloodmeal was tested twice using a direct ELISA technique, adapted from Service *et al*. (1986), for traces of human IgG, chicken IgG, and cow, goat and sheep IgG combined. Plates were read at 490 nm with an ELISA reader. A test result was considered positive if the optical density (OD) was greater than double the standard deviation (SD) above the mean OD of four replicates of a negative control. Among very few examples of multiple feeding, mosquitoes that had fed on both human and animal hosts were classified as human-fed.

Statistical analysis

For each village, the geometric mean (GM) number of female mosquitoes collected per catching effort was calculated for indoor and outdoor-resting mosquitoes, respectively, both before and after intervention, using the formula:

$$GM = \exp\left(\frac{\sum \ln(x_i + 1)}{n}\right) - 1$$

Comparisons of the mosquito numbers were made using the Wilcoxon paired-sample test (Wilcoxon signed rank test) (Zar, 1996). To correct for variation in mosquito numbers between the two years 1994 and 1995, the GM of mosquitoes in the control villages before (c_1) and after (c_2) intervention was used to adjust the mosquito numbers in the intervention villages before (i_1) and after (i_2) intervention. The relative reduction (R) in each intervention village was expressed as a percentage and calculated as follows (Curtis *et al.*, 1990):

$$R = 100\left(1 - \frac{i_2}{i_1 \frac{c_2}{c_1}}\right)\% = 100\left(1 - \frac{i_2 c_1}{i_1 c_2}\right)\%$$

The arithmetic mean relative reduction in the six village pairs and the 95% confidence interval (CI) was calculated for each mosquito species.

The human blood index (HBI) was calculated as the percentage of human-fed mosquitoes of all the bloodmeals identified. To obtain a sufficient sample size for analysis of feeding preferences, calculations were based on pooled samples of mosquito bloodmeals for intervention villages and control villages, respectively, in 1994 and likewise in 1995. Comparisons of feeding patterns were made between intervention and control villages, or between years, using a χ^2 test. Where cells had an expected frequency of less than five, Fisher's exact test was used (Zar, 1996).

Results

Species composition

A total of 19 847 female mosquitoes of filariasis vector species were caught during June–August 1994 and July–October 1995. The number of mosquitoes caught and the catching effort are listed in Table 1. Of those caught before intervention, 33.6% (3177) were *An. gambiae s.l.,* 30% (2835) *An. funestus* and 36.4% (3448) *Cx quinquefasciatus*. The *An. gambiae* complex was dominated by *An. gambiae s.s*. (98.5%) in August–September 1995, just after the rainy season, with only two specimens of *An. arabiensis* (1%) and one *An. merus* (0.5%) among the total of 190 *An. gambiae s.l.* identified by PCR.

Indoor-resting densities

For *An. gambiae*, *An. funestus* and *Cx. quinquefasciatus* these were not significantly different in the intervention and control villages in 1994. Comparison of the number of mosquitoes caught in the control villages in 1994 and 1995 demonstrated annual variation (Table 2), with 63% reduction of *An. gambiae s.l.* ($P < 0.05$) and a 37.2% reduction in the numbers of *Cx quinquefasciatus* ($P < 0.05$), but no significant difference in *An. funestus* density year on year.

The introduction of permethrin-impregnated bednets during June–July 1995 significantly reduced ($P < 0.05$) the number of indoor-resting *Anopheles*. The relative reduction in the intervention villages was 94.6% (CI: 85.9–103.4%) for *An. gambiae s.l.* and 96.7% (CI: 92.2–101.2%) for *An. funestus*. However, there was no significant decrease for indoor-resting *Cx quinquefasciatus*, with the relative density actually increasing between 1994 and 1995 for some villages (Table 2).

Outdoor-resting densities

For *An. funestus* and *Cx. quinquefasciatus* females in pit traps, these were unaffected by the intervention (Table 1), whereas *An. gambiae s.l.* decreased by 86.4% ($P < 0.05$).

Blood-meal identifications

ELISA identifications of 5435 mosquito bloodmeals were 96.9% successful (Table 3). Before intervention, *An. gambiae s.l.* was highly anthropophagic and endophilic, with a HBI of 99.2% in the control villages and 99.5% in the intervention villages. After distribution of the treated bednets (Fig. 1), the

intervention and control villages differed very significantly ($P < 0.001$). The HBI of indoor-resting *An. gambiae s.l.* was reduced to 85.7% in the intervention villages, with 9.5% feeding on ruminants and 4.8% feeding on chickens.

An. funestus was also highly endophilic and anthropophagic pre-intervention, when there were no bednets, with 99% feeding on humans in both village types. After intervention, the HBI of *An. funestus* indoors was significantly reduced to 52.2% ($P < 0.001$) with 43.5% feeding on ruminants and 4.3% feeding on chickens (Fig. 1).

The HBI of indoor-resting *Cx quinquefasciatus* was 90.2% in the control villages and 95.5% in the intervention villages before intervention. This difference was significant ($P < 0.01$): non-human bloodmeals were mostly from chickens. Post-

Table 1. Total and geometric mean (GM) numbers of female mosquitoes caught indoors (pyrethrum spray catches) and outdoors (from pit traps) in the intervention and non-intervention (control) villages, pre and post-intervention: June–August 1994 and July–October 1995.

	Pre-intervention 1994				Post-intervention 1995			
	Indoor		Outdoor		Indoor		Outdoor	
Species	Intervention	Control	Intervention	Control	Intervention	Control	Intervention	Control
An.gambiae s.l.	1291	1843	21	22	41	1539	2	9
An.funestus	1266	1513	33	23	224	4598	249	159
Cx quinquefasciatus	1256	2110	37	45	1863	1606	73	24
Total	3813	5466	91	90	2128	7743	324	192
No. of catches	66	70	43	44	209	93	112	78
GM/catch	41.4	59.4	1.2	1.0	2.5	51.7	1.4	0.9

Table 2. Mosquito population density in houses: geometric mean (GM) numbers of indoor resting female mosquitoes per house caught at daytime (pyrethrum spray catch) in the intervention (int.) and non-intervention (con.) villages pre-intervention 1994 and post-intervention 1995 and the percentage relative reduction (red.) in their abundance.

	Anopheles gambiae s.l.					*Anopheles funestus*					*Culex quinquefasciatus*				
	Pre-int. 1994		Post-int. 1995			Pre-int. 1994		Post-int. 1995			Pre-int. 1994		Post-int. 1995		
Village	Int.	Con.	Int.	Con.	Red.	Int.	Con.	Int.	Con.	Red.	Int.	Con.	Int.	Con.	Red.
pair					(%)					(%)					(%)
1	6.29	5.74	0.10	0.42	77.6	7.11	6.21	0.90	6.98	88.8	11.97	45.64	4.46	9.11	–86.7*
2	21.81	5.52	0.22	4.42	98.7	27.35	5.12	0.53	35.57	99.7	27.30	18.96	7.12	12.93	61.8
3	32.53	48.31	0.15	9.32	97.5	61.83	101.58	0.94	91.31	98.3	0.59	0.79	0.13	0.08	–113.5*
4	19.08	12.64	0.05	2.95	98.9	19.60	4.41	0.57	9.46	98.7	0.09	0.11	0.02	0.21	85.9
5	5.15	92.01	0.08	51.31	97.2	0.26	2.24	0.00	19.97	100.0	3.33	0.91	1.15	0.50	37.9
6	17.03	13.97	0.13	5.08	97.9	1.77	2.82	1.26	38.96	94.8	2.84	21.39	0.31	7.00	67.0
Mean	16.98	29.70	0.12	12.25	94.6	19.65	20.40	0.70	33.71	96.7	7.69	14.63	2.20	4.97	8.7
95%CI					85.9–103.4					92.2–101.2					–81.6–99.1

*Negative values represent less reduction for intervention villages than for non-intervention (control) villages, year on year.

Table 3. The human blood index (%) and the number of bloodmeals identified (in brackets) from mosquitoes caught indoors and outdoors in the intervention and non-intervention (control) villages in 1994 and 1995.

	Pre-intervention 1994				Post-intervention 1995			
	Indoor		Outdoor		Indoor		Outdoor	
Species	Intervention	Control	Intervention	Control	Intervention	Control	Intervention	Control
An. gambiae s.l.	99.5	99.2	44.4	50	85.7	99.8	–	80
	(626)	(647)	(9)	(10)	(21)	(461)	(0)	(5)
An. funestus	99	99	100	80	52.2	99	13.8	80
	(506)	(500)	(3)	(5)	(92)	(698)	(80)	(15)
Cx quinquefasciatus	95.5	90.2	100	50	14.4	93.1	66.7	100
	(332)	(440)	(1)	(2)	(458)	(350)	(3)	(3)

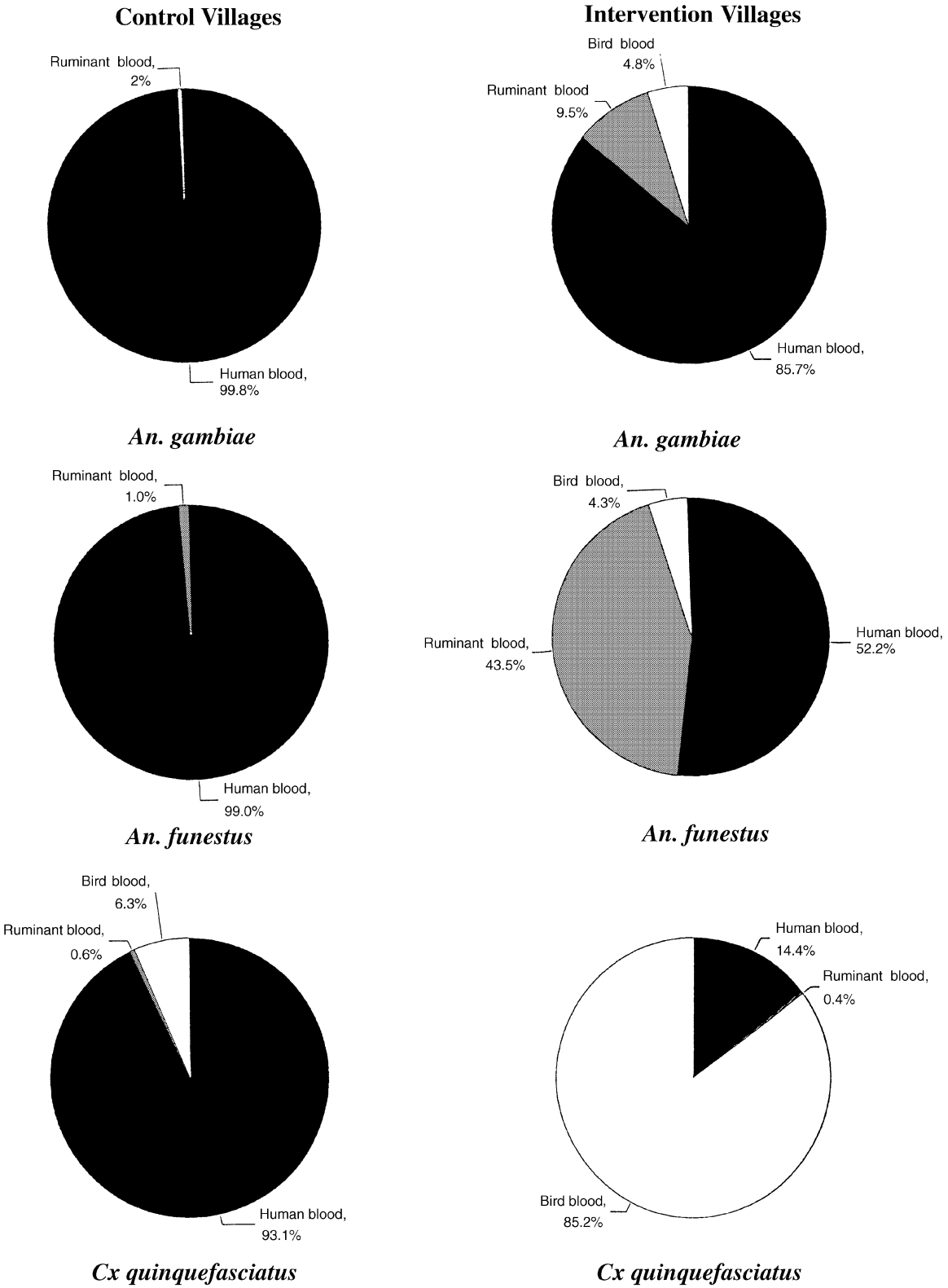

Fig. 1. Bloodmeal indices of indoor-resting mosquitoes after introduction of impregnated bednets. The distribution is shown for control villages (left column) and intervention villages (right column) in 1995.

intervention there was a marked shift of *Cx quinquefasciatus* away from feeding on humans: the HBI of indoor-resting *Cx quinquefasciatus* was reduced to 14.4% ($P < 0.001$) in the intervention villages with nearly all (85.2%) feeding on chickens and 0.4% feeding on ruminants.

Sample sizes of bloodfed *An. gambiae s.l.* ($n = 5$) and *Cx quinquefasciatus* ($n = 3$) from pit traps were too small for analysis (Table 3). In control villages, the outdoor-resting *An. funestus* had a HBI of 80%, with 20% feeding on ruminants in both 1994 ($n = 5$) and 1995 ($n = 15$). In intervention villages, the outdoor-resting *An. funestus* had a HBI of 100% in 1994 ($n = 3$) but, after distribution of the bednets in 1995, the HBI outdoors was reduced to 13.8% ($n = 80$) with 86.3% feeding on ruminants. This HBI difference between intervention and control villages in 1995 was highly significant ($P < 0.001$).

Discussion

The predominant indoor-resting mosquitoes in the study area were *An. gambiae s.l., An. funestus* and *Cx quinquefasciatus*, all being highly anthropophilic. For the anophelines this corresponds with the results of Mbogo *et al*. (1993) from Kilifi district on the coast north of Mombasa. In the months just after distribution of the treated bednets the indoor-resting density of *An. gambiae s.l.* was reduced to only about 5% of that in the comparison area. This decline is of the same magnitude as that reported from similar trials in Tanzania, East Africa (Magesa *et al*., 1991) and Guinea Bissau, West Africa (Jaenson *et al*., 1994) and demonstrates the effectiveness of impregnated bednets against *An. gambiae s.l.* Suppressed numbers of indoor-resting *An. gambiae s.l.* in rooms with treated nets could result from both killing and deterrent effects (Rozendaal, 1989; Lindsay *et al*., 1991). The associated reduction of outdoor-resting *An. gambiae s.l.*, found also by Magesa *et al*. (1991) in Tanzania, suggests an overall reduction in the *An. gambiae s.l.* population, rather than increased exophily. As nearly all specimens of the *An. gambiae* complex identified from the villages were *An. gambiae s.s.*, it was impossible to detect any differential effect of impregnated bednets on sibling species of the *An. gambiae* complex, as demonstrated by Mbogo *et al*. (1996).

The introduction of treated bednets was associated with a 96.7% relative reduction of indoor-resting *An. funestus* without a corresponding increase in the number resting outdoors, but with a much higher proportion of both indoor and outdoor-resting *An. funestus* feeding on animals. Thus, the intervention reduced the overall population density of *An. funestus* and diverted some of the remaining females from feeding on humans to feeding mainly on ruminants and occasionally on birds. The much higher proportion of animal-fed outdoor-resting '*An. funestus*' indicates a shift among the outdoor-resting females from endophagy on humans to exophagy on animals. Mosquitoes were possibly deterred from entering houses with treated nets, or exited before feeding because of irritation after contact with treated nets indoors (Rozendaal, 1989). Alternatively, this apparent change in feeding behaviour may be due to relatively more zoophilic members of the *An. funestus* group being present, the most likely being *An. parensis* Gillies (Gillies & Furlong, 1964) and *An. rivulorum* (Wilkes *et al*., 1996).

Impregnated bednets did not reduce the number of indoor-resting *Cx quinquefasciatus*, as may be expected, because this species is less susceptible than anophelines to permethrin (Hossain *et al*., 1989; Magesa *et al*., 1991; Wu Neng *et al*., 1991; Curtis *et al*., 1996) and other insecticides generally (Brown & Pal, 1971). However, the permethrin-impregnated nets induced a marked shift from anthropophagy to ornithophagy, with 85.2% of *Cx quinquefasciatus* females feeding on birds after treated nets were introduced, compared to 6.3% in the control villages. This shift to feeding mainly on chickens, that are often found indoors in this area, shows how a large population of *Cx quinquefasciatus* is maintained when access to human bloodmeals is restricted. The mosquito behaviour related to this feeding shift has not been observed. *Culex quinquefasciatus* females might be repelled before reaching the nets, or irritated after making contact with the treated surface of the nets and then diverted to feeding on chickens. If they are repelled before landing, changes in the type of insecticide or solvent might make the nets more effective in killing *Cx quinquefasciatus*, as demonstrated for anophelines by Lindsay *et al*. (1991) and Pleass *et al*. (1993). On the other hand, Curtis *et al*. (1996) showed in experimental hut trials using nets impregnated with various formulations of insecticides at dosages giving effective control of anopheline mosquitoes, that none of them effectively raised *Cx quinquefasciatus* mortality. Even so, our results suggest that permethrin-impregnated bednets would greatly reduce filariasis transmission by *Cx quinquefasciatus* because of the strong diversion from human-biting to bird-biting behaviour.

By expressing our data for each mosquito species in terms of vectorial capacity (Garrett-Jones, 1964), we may quantify the relative potential of each vector in Kwale villages with and without intervention, before (1994) and after (1995) the introduction of permethrin-impregnated bednets. As we did not measure the mosquito parous rates we cannot estimate the daily probability of mosquito survival (p), the most powerful component of vectorial capacity. Insecticidal bednets would undoubtedly make a considerable impact on mosquito survival, but we have only crude estimates of mosquito density (m) to account for this. Also we have the human blood index (HBI) from which to derive relative values for mosquito man-biting habits (a), assuming a standard mean duration of the gonotrophic cycle in days. Hence, Table 4 compares partial vectorial capacity values for each type of mosquito. The expression ma^2 represents the relative density of mosquitoes that bite humans repeatedly, based on standard sampling from daytime resting sites of mosquitoes indoors and outdoors, without knowing the actual biting rates of mosquitoes on humans vs. other hosts, and not knowing what proportions of mosquito populations rest outdoors (in pits, etc.) or in houses during the day. As the expression ma^2 is based on mosquito density and host choice, however, it can be used to compare vector potential.

Vector potential values for control (non-intervention) villages correspond well with pre-intervention values for bednet

Table 4. Partial vectorial capacity factors for three types of mosquitoes in Kwale villages before (1994) and after (1995) introduction of permethrin-impregnated bednets. Densities are derived from Table 1 as arithmetric means per catch.

		Control (non-intervention)			Bednets (intervention)		
Species and site	Factor	Pre (1994)	Post (1995)	Mean	Pre (1994)	Post (1995)	Change (%)
An. gambiae							
Indoors	Density (m)	26.3	16.6	21.5	19.6	0.20	–99
	HBI (a)	0.992	0.998	0.995	0.995	0.86	–14
	ma^2 (IN)	25.9	16.5	21.3	19.4	0.15	–99
Outdoors	Density (m)	0.50	0.12	0.31	0.49	0.02	–96
	HBI (a)	0.50	0.80	0.65	0.44	(0.44)	?
	ma^2 (OUT)	0.13	0.08	0.13	0.095	0.004	–96
	ma^2 (mean)	13.0	8.3	10.7	9.7	0.08	–99
An. funestus							
Indoors	Density (m)	21.6	49.4	35.5	19.2	1.07	–94
	HBI (a)	0.990	0.990	0.990	0.990	0.52	–47
	ma^2 (IN)	21.2	48.4	34.8	18.8	0.29	–98
Outdoors	Density (m)	0.52	2.04	1.28	0.77	2.22	188
	HBI (a)	0.80	0.80	0.80	1.00	0.14	–86
	ma^2 (OUT)	0.33	1.31	0.82	0.77	0.044	–94
	ma^2 (mean)	10.8	24.9	17.8	9.8	0.17	–98
Cx quinquefasciatus							
Indoors	Density (m)	30.1	17.3	23.7	19.0	8.9	–53
	HBI (a)	0.902	0.931	0.917	0.955	0.14	–85
	ma^2 (IN)	24.5	15.0	19.9	17.3	0.19	–99
Outdoors	Density (m)	1.02	0.31	0.67	0.86	0.65	–24
	HBI (a)	0.50	1.00	0.75	1.00	0.67	–33
	ma^2 (OUT)	0.26	0.31	0.38	0.86	0.29	–66
	ma^2 (mean)	12.4	7.7	10.1	9.1	0.24	–97

villages: overall mean values of ma^2 (Table 4) were 10.7 and 9.7 for *An. gambiae s.l.*, 17.8 and 9.8 for *An. funestus* and 10.1 and 9.1 for *Cx quinquefasciatus*, showing similar transmission potentials of all three filariasis vectors in Kwale villages. The impact of insecticidal bednets was estimated to reduce the vector potential by 99% for *An. gambiae s.l.*, by 98% for *An. funestus* and by 97% for *Cx quinquefasciatus*, not including the likely reduction of mosquito survival rates that would make an even greater impact on vectorial capacity (*sensu* Garrett-Jones, 1964). It is interesting to note that the impact of bednets on *Cx quinquefasciatus* was not so much on density as on the HBI, due to deviation from humans to biting of birds. The impact on *An. gambiae s.l.* was almost entirely due to density reduction, which would give enhanced suppression of vectorial capacity by reduction of p^n, the probability of vector survival to the infective stage. For '*An. funestus*' it is difficult to interpret the mixture of results associated with bednets, as the density dropped by 96.7% indoors but rose by 188% outdoors, while HBI fell by 47% and 86% indoors and outdoors, respectively. As already mentioned above, the explanation could be a shift of sibling species among the *An. funestus* group, or a shift of host preference from man to cattle associated with greater exophagy, or both. Pending more understanding of these entomological factors, we consider that permethrin-impregnated bednets are an effective and appropriate method of personal protection against vectors of *W. bancrofti* as well as malaria (Curtis, 1990) in East Africa.

Acknowledgements

The authors wish to thank the staff members of the Division of Vector Borne Diseases, Kenyan Ministry of Health, for their dedication and support for the project. A special thanks goes to technician Samuel Ruto for his very good and persistent field and laboratory work and to Philip Kange′the for his entomological support. Fiona Shenton (DBL) and The State Serum Institute in Copenhagen kindly helped with the PCR analysis, and Mikael Münster-Swendsen (University of Copenhagen) was supportive during the working process. We also greatly wish to thank Henry Madsen (DBL) for his help with data processing and Steve Lindsay (DBL) for comments on this paper.

We thank the Danish Bilharziasis Laboratory for providing the financial and practical support for this project; Axel Hemmingsens Fond for financial support for the second field trip; AgrEvo Environmental Health for providing insecticide and Siamdutch Mosquito Netting Co. for impregnating and numbering the bednets. This paper is published with kind permission of the Director of Medical Services of the Government of Kenya.

References

Alonso, P.L., Lindsay, S.W., Armstrong Schellenberg, J.R.M., Keita, K., Gomez, P., Shenton, F.C., Hill, A.G., David, P.H., Fegan, G., Cham, K.

& Greenwood, B.M. (1993) A malaria control trial using insecticide-treated bed nets and targeted chemoprophylaxis in a rural area of The Gambia, West Africa. 6. The impact of the interventions on mortality and morbidity from malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87** (Suppl. 2), 37–44.

Binka, F.N., Kubaje, A., Adjuik, M., Williams, L.A., Lengeler, C., Maude, G.H., Armah, G.E., Kajihara, B., Adiamah, J.H. & Smith, P.G. (1996) Impact of permethrin-impregnated bednets on child mortality in Kassena-Nankana district, Ghana: a randomized controlled trial. *Tropical Medicine and International Health*, **1**, 147–154.

Brown, A.W.A. & Pal, R. (1971) *Insecticide Resistance in Arthropods.* 2nd edn. World Health Organization Monograph Series. WHO, Geneva.

Choi, H.W., Breman, J.G., Teutsch, S.M., Liu, S., Hightower, A.W. & Sexton, J.D. (1995) The effectiveness of insecticide-impregnated bednets in reducing cases of malaria infection: a meta-analysis of published results. *The American Journal of Tropical Medicine and Hygiene*, **52**, 377–382.

Curtis, C.F. *et al.* (1990) Impregnated bednets and curtains against malaria mosquitoes. *Appropriate Technology in Vector Control* (ed. by C. F. Curtis), pp. 5–46. CRC Press, Boca Raton, Florida.

Curtis, C.F., Myamba, J. & Wilkes, T.J. (1996) Comparison of different insecticides and fabrics for anti-mosquito bednets and curtains. *Medical and Veterinary Entomology*, **10**, 1–11.

Edwards, F.W. (1941) *Mosquitoes of the Ethiopian Region. III. Culicine Adults and Pupae.* British Museum (Natural History), London.

Evengård, B., Linder, E. & Lundbergh, P. (1988) Standardization of a filter-paper technique for blood sampling. *Annals of Tropical Medicine and Parasitology*, **82**, 295–303.

Garrett-Jones, C. (1964) The human blood index of malaria vectors in relation to epidemiological assessment. *Bulletin of the World Health Organization*, **30**, 241–261.

Gillies, M.T. & De Meillon, B. (1968) *The Anophelinae of Africa South of the Sahara* (2nd edn). Publications of the South African Institute for Medical Research, No. 54, Johannesburg.

Gillies, M.T. & Furlong, M. (1964) An investigation into the behaviour of *Anopheles paarensis* on the Kenya coast. *Bulletin of Entomological Research*, **55**, 1–22.

Hossain, M.I., Curtis, C.F. & Heekin, J.P. (1989) Assays of permethrin-impregnated fabrics and bioassays with mosquitoes. *Bulletin of Entomological Research*, **79**, 299–308.

Jaenson, T.G.T., Gomes, M.J. Barreto dos Santos, R.C., Petrarca, V., Fortini, D., Evora, J. & Crato, J. (1994) Control of endophagic *Anopheles* mosquitoes and human malaria in Guinea Bissau, West Africa by permethrin-treated bed nets. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **88**, 620–624.

Lindsay, S.W., Adiamah, J.H., Miller, J.E. & Armstrong, J.R.M. (1991) Pyrethroid-treated bednet effects on mosquitoes of the *Anopheles gambiae* complex in The Gambia. *Medical and Veterinary Entomology*, **5**, 477–483.

Lindsay, S.W., Alonso, P.L., Armstrong Schellenberg, J.R.M., Hemingway, J., Adiamah, J., Shenton, F.C., Jawara, M. & Greenwood, B.M. (1993) A malaria control trial using insecticide-treated bednets and targeted chemoprophylaxis in a rural area of The Gambia, West Africa. 7. Impact of permethrin-impregnated bed nets on malaria vectors. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87** (Suppl. 2), 45–51.

Magesa, S.M., Wilkes, T.J., Mnzava, A.E.P., Njunwa, K.J., Myamba, J., Kivuyo, M.D.P., Hill, N., Lines, J.D. & Curtis, C.F. (1991) Trial of pyrethroid-impregnated bednets in an area of Tanzania holoendemic for malaria. 2. Effects on the malaria vector population. *Acta Tropica*, **49**, 97–108.

Mbogo, C.N.M., Baya, N.M., Ofulla, A.V.O., Githure, J.I. & Snow, R.W. (1996) The impact of permethrin-impregnated bednets on malaria vectors on the Kenyan coast. *Medical and Veterinary Entomology*, **10**, 251–259.

Mbogo, C.N.M., Kabiru, E.W., Muiruri, S.K., Nzovu, J.M., Ouma, J.H., Githure, J.I. & Beier, J.C. (1993) Bloodfeeding behavior of *Anopheles gambiae s.l.* and *Anopheles funestus* in Kilifi district. Kenya. *Journal of the American Mosquito Control Association*, **9**, 225–227.

Mosha, F.W. & Petrarca, V. (1983) Ecological studies on *Anopheles gambiae* complex sibling species on the Kenyan coast. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **77**, 344–345.

Mutinga, M.J., Renapurkar, D.M., Wachira, D.W., Mutero, C.M. & Basimike, M. (1992) Evaluation of the residual efficacy of permethrin-impregnated screens used against mosquitoes in Marigat, Baringo district, Kenya. *Tropical Medicine and Parasitology*, **43**, 277–281.

Nevill, C.G., Some, E.S., Mung'ala, V.O., Mutemi, W., New, L., Marsh, K., Lengeler, C. & Snow, R.W. (1996) Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. *Tropical Medicine and International Health*, **1**, 139–146.

Pleass, R.J., Armstrong, J.R.M., Curtis, C.F., Jawara, M. & Lindsay, S.W. (1993) Comparison of permethrin treatment for bednets in The Gambia. *Bulletin of Entomological Research*, **83**, 133–140.

Rozendaal, J.A. (1989) Impregnated mosquito nets and curtains for self-protection and vector control. *Tropical Diseases Bulletin*, **86**, R1–R41.

Scott, J.A., Brogdon, W.G. & Collins, F.H. (1993) Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *The American journal of Tropical Medicine and Hygiene*, **49**, 520–529.

Service, M.W. (1976) *Mosquito Ecology.* Applied Science Publishers, London.

Service, M.W., Voller, A. & Bidwell, D.E. (1986) The enzyme linked immunosorbent assay (ELISA) test for the identification of blood-meals of haematophagous insects. *Bulletin of Entomological Research*, **76**, 321–330.

Southgate, B.A. (1992) Intensity and efficiency of transmission and the development of microfilaraemia and disease: their relationship in lymphatic filariasis. *Journal of Tropical Medicine and Hygiene*, **95**, 1–12.

White, G.B. (1971) Studies on transmission of Bancroftian filariasis in north-eastern Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **65**, 819–829.

Wijers, D.J.B. (1977) Bancroftian filariasis in Kenya. 4. Disease distribution and transmission dynamics. *Annals of Tropical Medicine and Parasitology*, **71**, 451–463.

Wilkes, T.J., Matola, Y.G. & Charlwood, J.D. (1996) *Anopheles rivulorum*, a vector of human malaria in Africa. *Medical and Veterinary Entomology*, **10**, 108–110.

Wu Neng, Xia, Y., Chen, D.H. & Huang, F.M. (1991) Laboratory evaluation of efficacy of bednets impregnated with pyrethroids. *Journal of the American Mosquito Control Association.*, **7**, 294–298.

Zar, J.H. (1996) *Biostatistical Analysis*, 3rd edn., Prentice-Hall, Upper Saddle River, New Jersey.

Accepted 12 August 1997