Acta Tropica, 49(1991)97–108
Elsevier

ACTROP 00137

Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria Part 2. Effects on the malaria vector population

S.M. Magesa[1], T.J. Wilkes[1,2], A.E.P. Mnzava[1], K.J. Njunwa[1], J. Myamba[1], M.D.P. Kivuyo[1], N. Hill[2], J.D. Lines[1,2] and C.F. Curtis[2]

[1] *Amani Medical Research Centre, Amani, Tanzania, and* [2]*London School of Hygiene and Tropical Medicine, London, U.K.*

(Received 26 July 1990; accepted 30 October 1990)

The malaria vector population consisted mainly of *Anopheles gambiae* s.s. with a small contribution from *An. funestus* and *An. rivulorum*. The mosquitoes coming to bite in bedrooms were monitored with light traps set beside untreated bednets. When impregnated bednets were provided for all the other beds in a village the *Anopheles* populations declined but the *Culex quinquefasciatus* populations were unaffected. The survival of *An. gambiae* (as measured by the mean number of ovarian dilatations) and the sporozoite rate declined following introduction of the nets and the estimated sporozoite inoculation rates into people not under their nets declined by more than 90%. The net introductions caused sharp declines in the number of mosquitoes resting indoors, but the evidence was inconclusive regarding diversion to outdoor resting, animal biting, earlier biting or outdoor biting. DDT spraying greatly reduced the *Anopheles* populations.

Key words: *Anopheles gambiae*; *Anopheles funestus*; *Anopheles rivulorum*; *Culex quinquefasciatus*; Bednets, impregnated; Light traps; Sporozoite inoculation rate; Ovarian dilatations

Introduction

In experimental huts, pyrethroid impregnated bednets have been shown to kill some mosquitoes, to prevent others from feeding on humans and to drive mosquitoes out of houses (Darriet et al., 1984; Lines et al., 1987). The intention in introducing widespread net impregnation is to exploit these various effects to improve the personal protection given by nets, even if they are torn, and to kill large numbers of mosquitoes attracted to nets and thereby to reduce the vectorial capacity of the local mosquito population even for those people not under nets (Curtis et al., 1990).

In Papua New Guinea, Charlwood and Graves (1987) showed that permethrin impregnation of nets caused marked reductions in (i) the number of *Anopheles farauti* caught biting humans, (ii) the daily survival of the mosquitoes, (iii) the estimated

Correspondence address: C.F. Curtis, London School of Hygiene and Tropical Medicine, London WC1E 7HT, U.K.

total vector population in the village, (iv) the number resting in houses, (v) the proportion blood fed, (vi) the proportion of the bloodmeals from humans. Furthermore, the time of blood feeding was shifted to earlier in the night, apparently because mosquitoes returning from oviposition could not easily blood feed the same night because of the presence of the nets, and therefore delayed their next meal until early the following night.

In Burkina Faso, Carnevale et al. (1988) showed reductions in the numbers of *Anopheles funestus* bites per man per night following introduction of deltamethrin impregnated nets. There were also reductions in the percentages of *Anopheles gambiae* and *An. funestus* which were parous and in their sporozoite rate. Multiplying the sporozoite rate by the number of bites indicated 90% reductions in the sporozoite inoculation rates (numbers of infective bites per night per person not under a net).

In The Gambia, Lindsay et al. (1989) estimated the personal protection conferred by permethrin impregnated nets by fitting exit traps to windows and recording the numbers of mosquitoes fed on human blood and captured in the traps and in rooms. A 90% reduction was found after net treatment with permethrin.

In China, Li Zuzi and Lu Baolin (in Curtis et al., 1990) reported several studies showing reductions in outdoor biting rates on humans and in mosquitoes captured landing on nets following their treatment with deltamethrin.

In the present study, entomological work concentrated on attempting to measure the effects of mass mosquito killing on the vectorial capacity of the populations of *An. gambiae* s.s. and *An. funestus*. The biting population was estimated from indoor light trap catches in rooms equipped with untreated nets (Lines et al., 1991a). There is a good correlation of such catches with human biting catches on adjacent nights, and three light traps are required to catch as many as a team of two catchers. The age structure and sporozoite rates in light traps correlate with those in human biting catches or indoor resting catches and it was therefore considered justifiable to use light traps as the main method of monitoring the biting vector population during this study.

Catches of mosquitoes resting in houses and in pit traps were compared in an attempt to detect diversion of mosquitoes out of houses. The origin of the bloodmeals in mosquitoes from the pits was determined in order to detect any diversion of mosquitoes from human to animal feeding.

Materials and Methods

The study area, malaria epidemiology and intervention methods have been described by Njunwa et al. (1991).

Mosquito collections were made in each village every two weeks. Three houses were selected in each village for regular use of CDC miniature light traps. These were hung close to bednets which remained untreated throughout the trial. The householder was responsible for connecting the trap to a rechargeable battery at dusk, tying the trap bag at dawn and disconnecting the battery.

To relate the trap catches to human biting rates, all night human landing catches were carried out on certain nights adjacent to those in which light traps were set and in the same houses that were used for trapping (Lines et al., 1991a). The mosquitoes caught were placed in a different cup for each hour so that the hourly pattern of biting was revealed. To assess outdoor biting before the villagers went to bed, outdoor human biting catches were carried out from 18:30 to 21:30 h.

Catches were made of morning resting mosquitoes in six designated houses in each village. These houses were not the same as those used for the light traps. In the brick-built houses of Mlingano and Umba, the roofs were relatively high and the pyrethrum spray method was used. In the traditional villages of Kumbamtoni, Mindu and Mng'aza, it was possible to reach the ceilings and the catches were made with torches and aspirators for 30 person-minutes per house.

Three pit traps, about 2 m deep, were dug in each village and collections were made from horizontal 'tunnels' dug in the four walls of each (Service, 1976).

At the Ubwari Field Station at Muheza, the mosquito catches were sorted by species, sexed and counted. Female anophelines brought back alive were dissected and scored for parity by the Detinova method, for the number of ovarian dilatations by the Polovodova method (Detinova, 1962) and for sporozoites visible in the salivary glands. Female anophelines found dead in the light traps were stored dry over silica gel and later the heads and thoraces were tested for presence of *Plasmodium falciparum* sporozoites by the ELISA method (Burkot et al., 1984).

Blood fed mosquitoes from the pit traps were squashed onto filter paper and the origin of the blood meal identified later by the ELISA method of Service et al. (1986). Data on parity, number of ovarian dilatations and sporozoites from mosquitoes caught in the pit traps are excluded from the tables presented because it was found that these mosquitoes tend to be significantly younger than those caught biting or resting indoors (Lines et al., 1991b).

A proportion of the *An. gambiae* s.l. collected were identified to sibling species by electrophoresis (Mnzava and Kilama, 1986).

Results and Discussion

Light trap and resting catches

Fig. 1 shows the results of the fortnightly light trapping in each village averaged over each quarter of the year. The regular pattern of maximal populations of *An. gambiae* between April and June (the main rainy season) was clearly evident. The *An. funestus* populations were smaller than those of *An. gambiae* and tended to be maximal between July and December.

In every case, the introduction of impregnated nets (dotted and dash-dot lines in Fig. 1) markedly reduced the trap catches of the anophelines compared with what would have been expected in the village and the season concerned. This cannot be explained by repellency because the traps were set in rooms with untreated nets. On the contrary, it indicates high mortality of the vector populations inflicted by the treated nets. The reduction in the catches following DDT treatment (dashed line in Fig. 1) cannot be so clearly interpreted as the traps were set in sprayed houses.

The right-hand diagrams in Fig. 1 show that, unlike the *Anopheles* populations, those of *Culex quinquefasciatus* were unaffected by the introduction of impregnated nets. It is known from the laboratory data of Hossain et al. (1989) that *Culex quinquefasciatus* is less susceptible to permethrin impregnated nets than *An. gambiae*. It has been argued that filariasis would be easier than malaria to control by impreg-

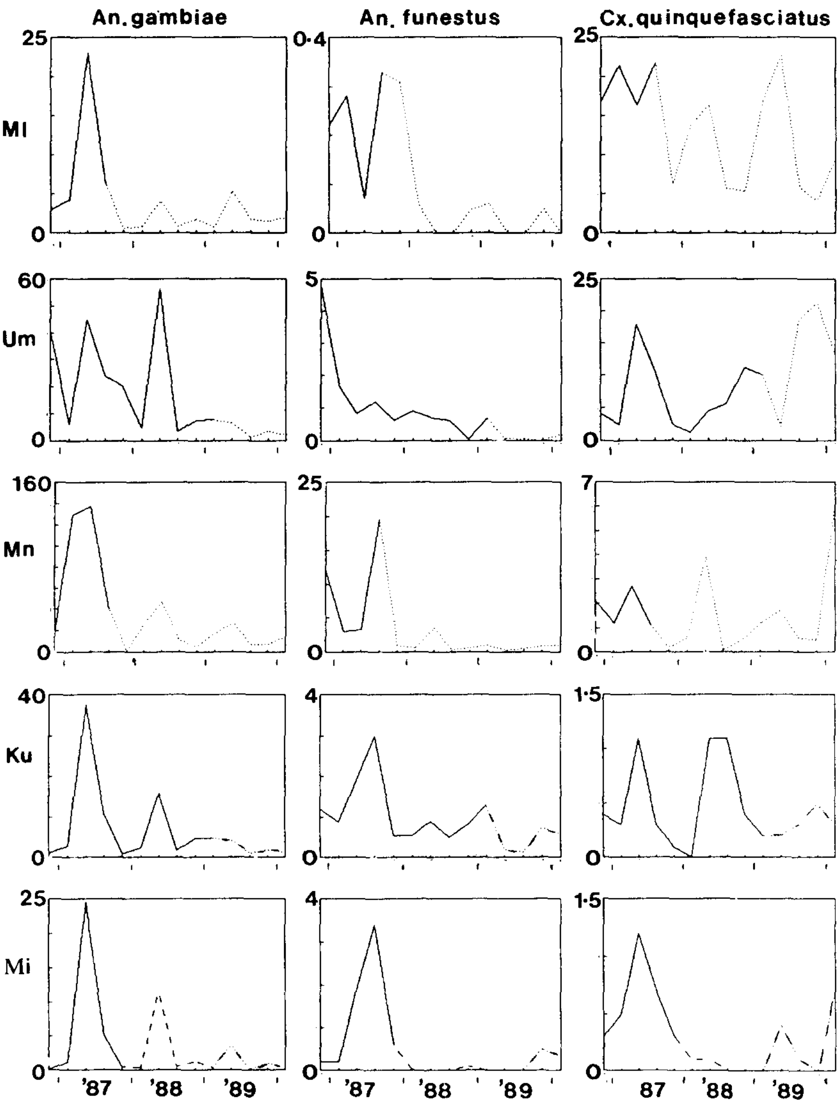

Fig. 1. Quarterly average light trap catches of the three main human biting mosquitoes in the five villages of Mlingano (Ml), Umba (Um), Mng'aza (Mn), Kumbamtoni (Ku) and Mindu (Mi). ——, no vector control; ......, permethrin impregnated nets; -·····-, lambdacyhalothrin inpregnated nets; - - -, DDT spraying. Note that the vertical scales are expanded or contracted to fit the maximum number of mosquitoes of the species and village concerned.

nated nets. However, in areas where filariasis is transmitted by *Cx. quinquefasciatus*, our data suggest that permethrin impregnated nets may not be an effective control method.

Fig. 2 shows, on log scales, the catches of *An. gambiae* resting in rooms against those caught resting in pit traps on the same days, before (open symbols) and after (filled symbols) introduction of treated nets. In Kumbamtoni and Mng'aza where the houses have traditional thatched roofs and hand catches were made, a large proportion of vectors caught were indoor resters, whereas the opposite was true for

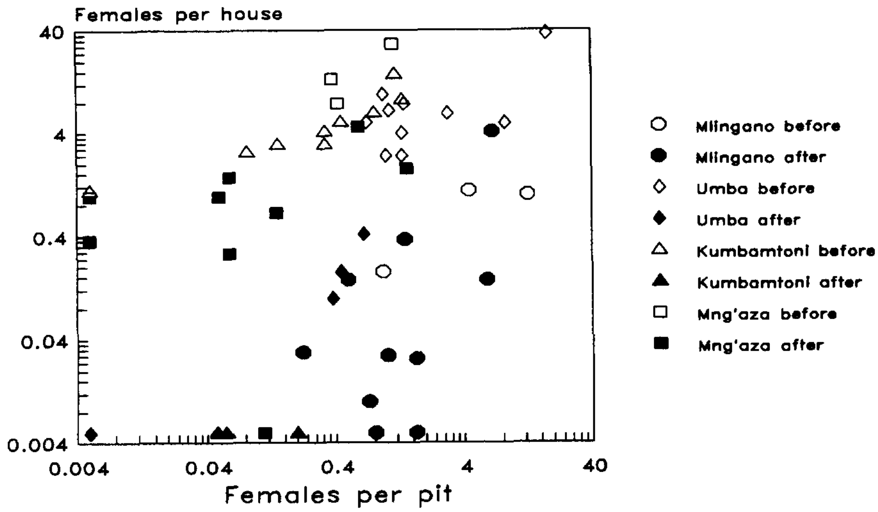

Fig. 2. *Y*-axis: mean pyrethrum spray catches of *An. gambiae* in the estate villages of Mlingano and Umba and resting catches with torches and aspirators in the traditional villages of Kumbamtoni and Mng'aza plotted on a log scale. *X*-axis: the pit trap catches in the corresponding quarters, also plotted on a log scale. 0.005 was added to each mean before plotting so as to allow zero scores to be accommodated. Open symbols, before vector control; filled symbols, after introduction of impregnated nets.

Mlingano and Umba where the houses have tiled roofs, and spray catches were made. After introduction of treated nets the indoor resting catches declined greatly. This can partly be explained by reduction in the population density, as shown by the light traps. The well known excito-repellent effect of permethrin might have been expected to shift the indoor/outdoor resting ratio, but Fig. 2 shows little evidence for this — on the whole the pit trap catches declined when the nets were introduced, rather than showing increases which might have been expected from the diversion of mosquitoes out of doors.

Electrophoresis of a sample of captured *An. gambiae* s.l. indicated that almost all were *An. gambiae* s.s as was expected in this part of the country (Mnzava and Kilama, 1986).

Tests on limited numbers of *An. gambiae* caught in resting catches in the treated villages indicated no evidence for any build up of resistance to the insecticides used.

Mosquito survival and sporozoite infection

Fig. 3 shows data for villages without vector control on proportions of female *An. gambiae* parous, mean number of ovarian dilatations and proportions found by dissection to carry sporozoites. There is a marked trend for all these measures of female age to be lowest during the population explosion of new emergence in the rains of April–June. The measures of mean adult age tend to be higher when conditions are less favourable for breeding. An exception was in January–March 1988 in Umba, when all these measures were relatively low.

Comparison of the estate villages (Umba and Mlingano, top of Fig. 3) with the traditional villages (Kumbamtoni, Mng'aza and Mindu, bottom of Fig. 3) shows a tendency for shorter mosquito survival in the former, perhaps because of the worse

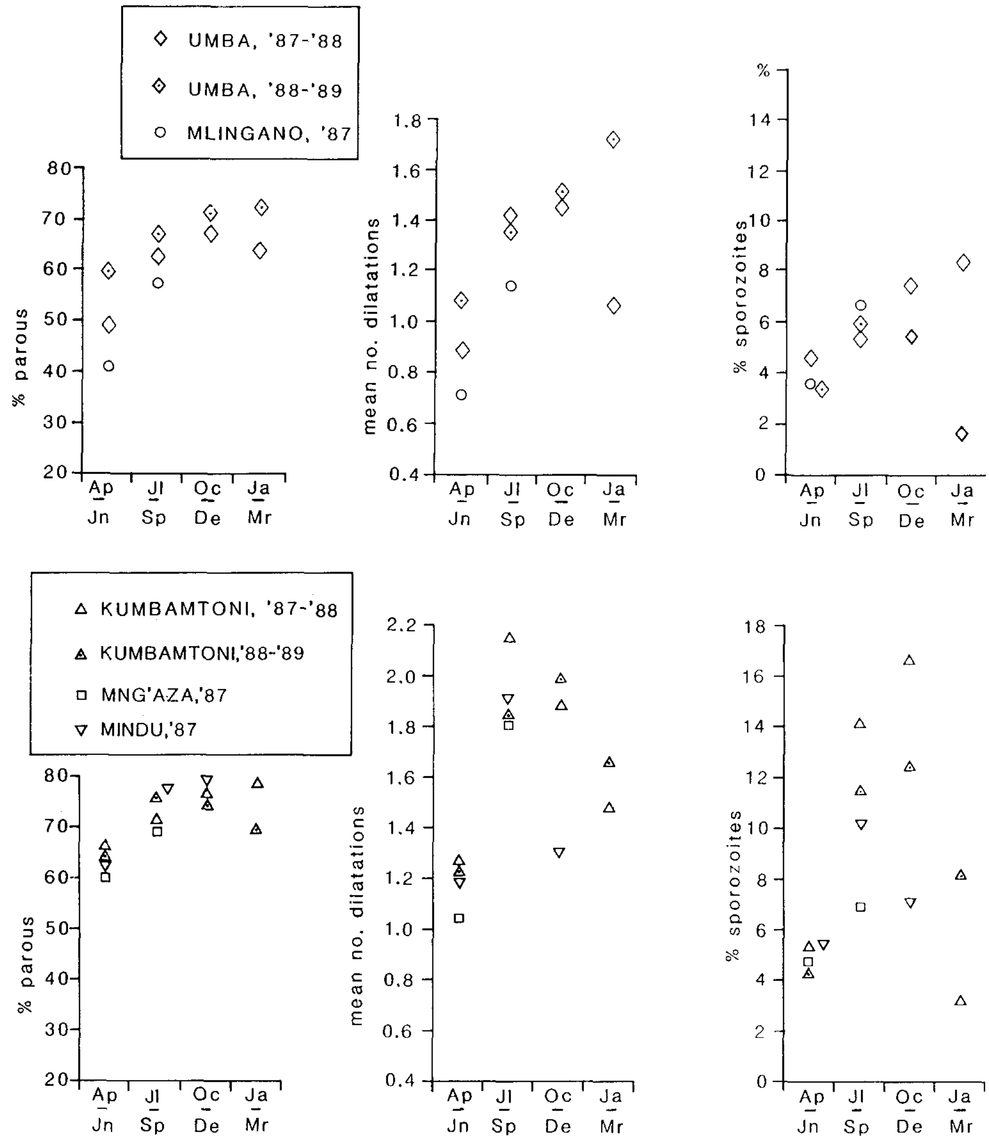

Fig. 3. Percentage of *An. gambiae* parous, mean number of ovarian dilatations and sporozoite infective rate in the four quarters of the year in the villages without vector control. Data from estate villages, with brick-built houses and tiled roofs, are shown above and those from traditional villages, with mud houses with palm thatch roofs, below.

conditions for mosquitoes under tiled roofs. Conversely, in the traditional village of Kumbamtoni all the measures of survival tended to be high and the sporozoite rate several times exceed 12%.

Fig. 4 shows the effects of introduction of permethrin impregnated nets or DDT spraying on the age structure of the *An. gambiae* population, as measured by number of ovarian dilatations. The reduction in vector survival is clearly apparent. As shown by Lines et al. (1991b) there is a very strong relationship between the higher dilatation categories and probability of carrying sporozoites. Thus the data in Fig. 4 suggest that vector control must have reduced the risks in the treated villages even for people not under treated nets.

Table 1 shows direct observations of the sporozoite rates in villages with and without vector control. In addition to the dissection data, there is a limited amount of data using the ELISA method. A weighted mean and a Mantel-Haenszel χ^2 test on all the data showed a slightly but significantly higher sporozoite rate by the ELISA than the dissection method. The difference was not so great as that reported by Beier et al. (1990). The mean sporozoite rate in *An. funestus* was very slightly, but just significantly higher than that in *An. gambiae*.

Although significant heterogeneities were found between the methods and vector species, the differences were small — apparently smaller than the seasonal effects shown in Fig. 3. In testing for the effects of vector control it therefore seemed best to pool the data for the two methods and two species but to keep those for each quarter of the year separate. Mantel-Haenszel χ^2 tests on this basis led to the conclusions that the introduction of permethrin impregnated nets in Mlingano, Umba and Mng'aza, and DDT spraying in Mindu, caused highly significant reductions in the sporozoite rates (Table 1). Between phases of the experiment when there was no change in vector control operations (e.g., between phases 2 and 3 in Mng'aza or phases 1 and 2 in Umba) the sporozoite rates generally did not change significantly. However, in Kumbamtoni the very high sporozoite rate seen in phase 1 declined significantly in phase 2, even though there was as yet no vector control in that village. When impregnated nets were introduced in phase 3 the observed sporozoite rate declined compared with phase 2, but not significantly so.

The data summarised in Fig. 1 from light traps may be multiplied by 1.5 to convert them to estimates of what the biting rates per person would have been, in the rooms concerned, in the absence of the traps and their associated untreated bednets (Lines et al., (1991a)). Multiplying these estimates for each quarter by the sporozoite rates

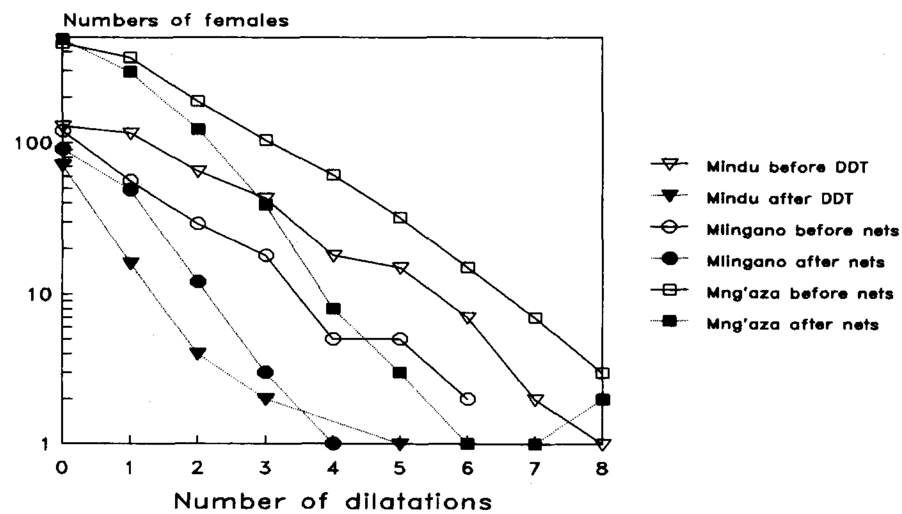

Fig. 4. Age structure of *An. gambiae* populations before (open symbols) and after (filled symbols) introduction of permethrin impregnated nets into Mlingano and Mng'aza and DDT spraying into Mindu.

TABLE 1

Comparison of the sporozoite rate (in % with No. tested in parentheses) as determined by dissection and by ELISA, in *An. gambiae* and *An. funestus* and in villages with or without vector control. Presence of permethrin impregnated nets is indicated by solid boxes, lambdacyhalothrin nets by boxes with broken lines and DDT spraying by a double box.

Village	Sp.	Method	Phase 1 (Sept'86–Sept'87)	Phase 2 (Oct'87–Apr'89)	Phase 3 (Apr'89–Mar'90)
Ml	*A.g.*	Diss.	5.3(244)	0.0(129)	1.0(100)
		ELISA	0.0(18)	0.0(29)	1.9(53)
Ml	*A.f.*	Diss.	0.0(6)	0.0(14)	0.0(1)
Um	*A.g.*	Diss.	5.2(555)	5.3(1328)	0.0(86)
		ELISA	7.5(133)	10.5(19)	3.9(76)
Um	*A.f.*	Diss.	1.9(53)	5.1(79)	0.0(5)
		ELISA	1.5(67)	0.0(4)	0.0(4)
Mn	*A.g.*	Diss.	5.5(1165)	1.6(1032)	2.3(429)
		ELISA	10.2(179)	3.8(503)	2.2(224)
Mn	*A.f.*	Diss.	3.5(286)	0.0(91)	4.9(81)
		ELISA	6.7(179)	4.3(46)	0.0(28)
Ku	*A.g.*	Diss.	10.0(550)	7.7(624)	2.5(40)
		ELISA	15.4(26)	7.0(86)	6.7(15)
Ku	*A.f.*	Diss.	9.8(204)	6.6(350)	0.0(6)
		ELISA	20.0(30)	4.2(48)	0.0(13)
Mi	*A.g.*	Diss.	7.6(370)	0.8(117[a])	0.0(22)
		ELISA	22.2(9)	5.1(84[a])	0.0(8)
Mi	*A.f.*	Diss.	7.5(282)	0.0(1[a])	–
		ELISA	3.8(26)	0.0(8[a])	0.0(2)

Village	Mean in phase 1	χ^2(M-H)	Mean in phase 2	χ^2(M-H)	Mean in phase 3
Ml	4.85%	←5.61*→	0.0	–	1.3%
Um	5.07%	←0.45 n.s.→	5.52%	←8.66**→	1.75%
Mn	5.75%	←36.35***→	2.21%	←0.37 n.s.→	2.30%
Ku	10.4%	←6.26*→	7.12%	←0.74 n.s.→	3.80%
Mi	7.57%	←9.03**→	2.38%	–	0.0%

[a]Data starts from Jan'88 when DDT was sprayed in Mindu.
Means and χ^2 (Mantel-Haenszel) comparisons:
Dissection = 5.08%, ELISA = 5.32%; χ^2(M-H) = 17.48***
An. gambiae = 5.02%, *An. funestus* = 5.54%; χ^2(M-H) = 4.99*

for the corresponding quarter yields estimates of the sporozoite inoculation rates (Table 2). In the six months before introduction of impregnated nets into Mng'aza the inoculation rate was as high as 7 infective bites per person per night, which gives some idea of the scale of the problem which has to be overcome in such villages if malaria is to be controlled.

In Mlingano and Mng'aza there are only adequate pre-treatment data on sporozoites for the period April–September 1987. Therefore, Table 2 compares the sporozoite inoculation rates before and after net introduction for these months in each year, for these two villages. In Umba and Kumbamtoni, net introduction was not until April 1989 and comparisons before and after can be made for all months of the year. It is concluded that the combined effect of reduction of numbers and sporozoite rates due to the impregnated nets was to reduce the risk of infection for someone outside a net by more than 90%. This measure of the 'mass effect' of the nets takes no account of the personal protection which they provide so long as people stay under them. In the case of DDT spraying at Mindu the sporozoite inoculation rate was estimated to have been reduced by 86%.

Sporozoites were found in three specimens of *An. rivulorum*, 2 out of 199 dissected from Umba and 1 out of 117 dissected from Mlingano. Although many individuals

TABLE 2

Estimated numbers of bites by the two vector species per person per night based on the light trap catches and their relationship to human biting catches. The sporozoite rates are based on the results of dissections and ELISA tests weighted for the numbers biting at the season concerned. Boxes indicate vector control methods as in Table 1.

Year	Bites /person /night	Sporozoite rate (%)	Sporo. inoc. /person /night	% reduction following vector control
Mlingano (Apr–Sept)				
'87	22.3	4.1	0.91	
'88	3.9	0.0	0.0	97
'89	4.6	0.28	0.013	
Umba (all months)				
'87–'88	36.3	4.9	1.8	
'88–'89	28.9	4.4	1.3	93
'89–'90	5.2	2.1	0.11	
Mng'aza (Apr–Sept)				
'87	150.7	4.8	7.3	
'88	47.0	1.8	0.86	91
'89	27.2	1.8	0.48	
Kumbamtoni (all months)				
'87–'88	21.5	7.8	1.7	
'88–'89	11.4	5.9	0.68	96
'89–'90	3.4	1.4	0.048	
Mindu (Apr-Dec)				
'87	18.1	6.7	1.2	
'88	2.0	2.5	0.17	86
'89	2.6	0.0	0.0	100

were collected from pits having fed on cattle (Table 3), some were found biting people outdoors in the early part of the evening. *An. rivulorum* has not hitherto been incriminated as a vector of malaria and we have yet to confirm that sporozoites found in it are of human origin. Further studies are underway to assess the importance of this species as a vector.

Blood meals and night biting catches

Apart from their effect in killing mosquitoes, it is intended that impregnated nets will confer a high level of personal protection on those who remain under them. It was expected that this might be reflected in diversion of mosquitoes from human to animal feeding and the best chance of picking up this effect would be in the pit trap collections. However, Table 3 shows no such effect; the great majority of feeds by *An. gambiae* were on humans even after introduction of the nets. On the other hand, *An. rivulorum* and/or *An. funestus* (which were not always clearly distinguished) fed predominantly on animals both before and after net introduction.

In The Gambia, Lindsay et al. (1989) used exit traps and resting collections to estimate the number of bites on humans indoors. Because of the many apertures in the houses in our area through which mosquitoes could exit, it was considered unlikely that this method would be reliable in our trial. Unfortunately, therefore, the only available estimate of the extent of the personal protection factor is that of about 90% by Lines et al. (1987) from preliminary work in experimental huts in another part of Tanzania. However, data on the anti-sporozoite antibody levels in the human population in the present trial are being processed by F. Esposito and A. Habluetzel and these may give an indication of the sporozoite inoculation rates actually experienced by the people.

Charlwood and Graves (1987) in Papua New Guinea showed that the use of permethrin impregnated bednets shifted the time of blood feeding of *An. farauti* to

TABLE 3

Blood meal identification on anophelines caught in pit traps before or after introduction of vector control with impregnated nets in Mlingano and Mng'aza and DDT spraying in Mindu. The blood meals were identified as from human (H), bovine (B) or goat (G) or were unidentifiable (-ve). Data for *An. funestus* and the closely related *An. rivulorum* are pooled as, on some occasions, these may not have been correctly separated.

		An. gambiae				*An. funestus/rivulorum*			
		H	B	G	-ve	H	B	G	-ve
Mlingano	- before	24	2	1	2	3	1	3	0
Mlingano	- after	23	3	1	8	1	7	0	1
Umba	- before	60	17	1	19	13[a]	127	10	17
Mng'aza	- after	7	1	0	0	1	2	0	0
Kumbamtoni	- before	7	0	0	0	1	0	0	1
Mindu	- after	7	0	0	1	–	–	–	–

[a]Plus one with both human and bovine blood.

earlier in the night. Table 4 shows a slight but statistically significant effect of this kind in *An. gambiae* in Mng'aza, but not in Mlingano. Conversely in Mlingano there was slight, but statistically significant, evidence for diversion towards outdoor biting, but there was no such effect in Mng'aza.

Conclusions

(i) Community wide use of impregnated nets reduced the *Anopheles* population density, their survival and sporozoite rate. The overall effect was to reduce the sporozoite inoculation rate into people outside nets by over 90%.
(ii) Fewer mosquitoes were caught resting in houses containing treated nets but pit trap catches did not show evidence for diversion to outdoor resting or animal biting.

Acknowledgements

We thank members of the entomology team from the Ubwari Field Station, and the householders who operated the light traps, for their conscientious work. We are

TABLE 4

Numbers of *An. gambiae* caught in human biting catches over the four quarters of the night inside houses, and in the first quarter outside. Data are derived from pooling several complete nights' collections before or after net introduction in Mng'aza and Mlingano; for each village the second line of data is from collections terminated at 21.30 h. N indicates the number of collections on which the data are based; n indicates the numbers caught. The percentages are based on the total inside collection. The mean biting times are derived from hour-by-hour data before pooling into the four quarters shown in the table. (The standard errors of the mean biting times are shown in minutes.)

Village	N	Before /after nets	Out 18.30–21.30	In 18.30–21.30		In 21.30–00.30		In 00.30–03.30		In 03.30–06.30		Mean biting time (SE)
			n	n	%	n	%	n	%	n	%	
Mn	2	before	11	2	0.59	61	18.2	115	34.2	158	47.0	02.54 (46)
Mn	5	before	15	20	–	–	–	–	–	–	–	–
Mn	8	after	29	24	4.5	86	16.2	211	39.7	211	39.7	02.35 (45)
Ml	3	before	8	6	6.7	25	28.1	34	38.2	24	27.0	01.44 (49)
Ml	4	before	10	11	–	–	–	–	–	–	–	–
Ml	7	after	20	4	7.0	19	33.3	22	38.6	12	21.0	01.13 (50)

χ^2 comparisons before and after net introduction gave the following results:

	Mng'aza	Mlingano
Between quarters of the night (d.f. = 3)	15.3 ($P<0.001$)	0.83 n.s.
Inside vs. outside (d.f. = 1) (including all those caught before 21.30 h)	0.001 n.s.	5.01 ($P<0.05$)

grateful to Prof. F. Esposito, and Drs. F. Collins and R. Wirtz for providing monoclonal antibodies. The project was supported financially by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, the Tanzanian Ministry of Health and the British Medical Research Council. This paper is published by permission of the Director of the Amani Centre and the Director General of the Tanzanian National Institute for Medical Research.

References

Beier, J.C., Perkins, P.V., Koros, J.K., Onyango, F.K., Gargan, T.P., Wirtz, R.A., Koech, D.K. and Roberts, C.R. (1990) Malaria sporozoite detection by dissection and ELISA to assess infectivity of Afrotropical *Anopheles*. J. Med. Ent. 27, 377–384.

Burkot, T.R., Williams, J.L., and Schneider, I. (1984) Identification of *Plasmodium falciparum*-infected mosquitoes by a double-antibody enzyme-linked immunosorbent assay. Am. J. Trop. Med. Hyg. 33, 783–788.

Carnevale, P., Robert, V., Boudin, C., Halna, J.-M., Pazart, L. Gazin, P., Richard, A., Mouchet, J. (1988) La lutte contre le paludisme par des moustiquaires imprégnées de pyréthrinoides au Burkina Faso. Bull. Soc. Pathol. Exot. 81, 832–846.

Charlwood, J.D. and Graves, P.M. (1987) The effects of permethrin bednets on the population of *Anopheles farauti* in coastal Papua New Guinea. Med. Vet. Ent. 1, 319–327.

Curtis, C.F., Lines, J.D., Carnevale, P., Robert, V., Boudin, C., Halna, J.-M., Pazart, L., Gazin, P., Richard, A., Mouchet, J., Charlwood, J.D., Graves, P.M., Hossain, M.I., Kurihara, T., Ichimori, K., Li Zuzi, Lu Baolin, Majori, G., Sabatinelli, G., Coluzzi, M., Njunwa, N.J., Wilkes, T.J., Snow, R.J. and Lindsay, S.W. (1990) Impregnated bed nets and curtains against malaria mosquitoes. In: C.F. Curtis (Ed.), Appropriate Technology in Vector Control, C.R.C., Boca Raton, FL.

Darriet, F., Robert, V., Tho Vien, N. and Carnevale, P. (1984) Evaluation of the efficacy of permethrin impregnated intact and perforated mosquito nets against vectors of malaria. WHO mimeographed document WHO/VBC 84.899.

Detinova, T.S. (1962) Age Grouping Methods in Diptera of Medical Importance. WHO Monogr. series No. 47, W.H.O., Geneva.

Hossain, M.I., Curtis, C.F. and Heekin, J.P. (1989) Assays of permethrin impregnated fabrics and bioassays with mosquitoes. Bull. Ent. Res. 79, 299–308.

Lindsay, S.W., Snow, R.W., Broomfield, G., Semega Janneh, M., Wirtz, R.A. and Greenwood, B.M. (1989) Impact of permethrin treated bednets on malaria transmission in The Gambia. Med. Vet. Ent. 3, 263–271.

Lines, J.D., Myamba, J. and Curtis, C.F. (1987) Experimental hut trials of permethrin impregnated nets and curtains against malaria vectors in Tanzania. Med. Vet. Ent. 1, 37–51.

Lines, J.D., Curtis, C.F., Wilkes, T.J. and Njunwa, K.J. (1991a) Monitoring human biting mosquitoes in Tanzania with light traps hung beside mosquito nets. Bull. Ent. Res., in press.

Lines, J.D., Wilkes, T.J. and Lyimo, E.O. (1991b) The relationship between age and sporozoite rate in *Anopheles gambiae* in Northern Tanzania. Parasitology, in press.

Mnzava, A.E.P. and Kilama, W.L. (1986) Observations on the distribution of the *Anopheles gambiae* complex in Tanzania. Acta Trop. 43, 277–282.

Njunwa, K.J., Lines, J.D., Magesa, S.M., Mnzava, A.E.P., Wilkes, T.J., Alilio, M., Kivumbi, K. and Curtis, C.F. (1991) Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 1. Operational methods and acceptability. Acta Trop. 49, 87–96.

Service, M.W., Voller, A. and Bidwell, D.E. (1986) The enzyme linked immunosorbent assay (ELISA) for the identification of blood meals of haematophagous insects. Bull. Ent. Res. 76, 321–330.

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