Tropical Medicine and International Health

VOLUME 3 NO 8 PP 619–631 AUGUST 1998

A comparison of use of a pyrethroid either for house spraying or for bednet treatment against malaria vectors

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Summary In an intensely malarious area in north-east Tanzania, microencapsulated lambdacyhalothrin was used in four villages for treatment of bednets (provided free of charge) and in another four villages the same insecticide was used for house spraying. Another four villages received neither intervention until the end of the trial but were monitored as controls. Bioassays showed prolonged persistence of the insecticidal residues. Light traps and ELISA testing showed reduction of the malaria vector populations and the sporozoite rates, leading to a reduction of about 90% in the entomological inoculation rate as a result of each treatment. Collections of blood fed mosquitoes showed no diversion from biting humans to biting animals. Incidence of re-infection was measured by weekly monitoring of cohorts of 60 children per village, after clearing pre-existing infection with chlorproguanil-dapsone. The vector control was associated with a reduction in probability of re-infection per child per week by 54–62%, with no significant difference between the two vector control methods. Cross-sectional surveys for fever, parasitaemia, haemoglobin and weight showed association of high parasitaemia with fever and anaemia and beneficial effects of each intervention in reducing anaemia. However, passive surveillance by resident health assistants showed no evidence for reduced prevalence of fever or parasitaemia. Net treatment consumed only about one sixth as much insecticide as house spraying and it was concluded that the former intervention would work out cheaper and nets were actively demanded by the villagers, whereas spraying was only passively assented to.

keywords impregnated bednets, house spraying, lambdacyhalothrin, Tanzania, malaria

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Introduction

There have been encouraging reductions of child mortality in Africa as a result of use of insecticide-treated bednets (see summary in Lengeler *et al*. 1996). It should not be overlooked, however, that equally impressive reductions in mortality were achieved in the Pare-Taveta scheme 40 years ago by spraying houses with dieldrin (see summary by Bradley 1991). It is often said that organizing routine house spraying is logistically impossible in much of tropical Africa, but organizing routine retreatment of nets is also demanding. Thus there was a need to compare the effectiveness and cost of these two methods.

In China and the Solomon Islands pyrethroid-treated nets were more effective than DDT house-spraying (Li & Lu 1989; Kere *et al*. 1996). Pyrethroids are preferable to DDT for house spraying (Curtis 1994). Sexton *et al*. (1994) compared permethrin-treated nets with lambdacyhalothrin house-spraying in Nigeria but detailed results have not yet been presented.

Lambdacyhalothrin is effective and more persistent at much lower doses than permethrin and appears to be less affected by washing (Curtis *et al*. 1996). We have therefore compared lambdacyhalothrin, in microencapsulated form, used for house spraying, with the same formulation used for bednet treatment. The study was carried out in several replicate villages near Muheza in north-east Tanzania, where malaria transmission is very intense. In separate trials a few years ago in this area, major reductions in entomological inoculation rate and in incidence of malaria infection were achieved with lambdacyhalothrin used for net treatment (Magesa *et al*. 1991; Msuya & Curtis 1991) or for house spraying (Matola *et al*. 1990). In these bednet trials effects on prevalence of malaria fever were much less clear-cut (Lyimo *et al*. 1991).

For studies on incidence of re-infection, sulfadoxine-pyrimethamine had previously been used to clear pre-existing parasitaemia (Msuya & Curtis 1991; Stich *et al.* 1994). However, in 1995 resistance was found to this drug combination in this area, but good clearance of parasitaemia one week after treatment was found with chlorproguanil-dapsone (Trigg *et al.* 1997). The latter combination was therefore used in the present trial.

Site, materials and methods

Site and organization of the trial

The trial was carried out in 12 villages, six of which were near Muheza, Tanga Region, on the road to Pangani which were the sites of malaria studies by Clyde & Shute (1957) and other later studies. In this humid area the vectors are *An. gambiae s.s.* and *An. funestus* (Mnzava & Kilama 1986; Magesa *et al.* 1991). The other six trial villages were 30 km further inland near Hale, where the climate is drier and there is an admixture of *An. arabiensis* in the vector population (White *et al.* 1972). These villages have been the site of primary health care activity instigated by the Pangani Falls Redevelopment Project. The village populations were censused and found to range between 971 and 2445, with an average of 17.9% aged less than 6 years. Data on mortality for the villages near Muheza are given by Salum *et al.* (1994).

The main rainy season is from April to June and populations of *An. gambiae s.l.* and malaria cases peak at this time, but there are perennial vector populations and malaria transmission.

Agreement to the trial was given after consultations with village authorities and mass meetings in each of the 12 villages. The inhabitants of those villages which did not receive nets during the trial were told that they would receive them at the end of it. Clearance, especially for the use of chlorproguanil dapsone, was given by the Ethical Committee of the Tanzanian National Institute for Medical Research.

Some baseline data were collected during 1995. Consideration was given to stratification of the villages prior to their assignment to nets, spraying or controls. However, no significant differences were found between villages in the baseline data on vector populations and malaria incidence, so it was decided to randomly assign two villages from Muheza and two from Hale to each of the treatments, leaving two villages in each area as controls (i.e. a total of 4:4:4, control:netted:sprayed villages). The interventions were introduced in 6 of the villages in December 1995 but, owing to logistical problems, spraying and provision of nets was delayed in two until February 1996. Until these villages had been treated, data from them were excluded from the post-intervention data presented.

Entomology

The village mosquito populations were monitored throughout 1995 and 1996 using monthly light trapping (Lines *et al.* 1991) in each village in five sentinel bedrooms which were provided with untreated bednets and not sprayed. After identification and counting, anopheline females were stored on silica gel for detection of *Plasmodium falciparum* circumsporozoite protein in the heads and thoraces by ELISA (Burkot *et al.* 1984) and, on a subsample, distinction between *An. gambiae s.s.* and *An. arabiensis* by PCR (Scott *et al.* 1993).

The personal protective effects of the interventions were monitored monthly in 1996 in each of the 12 villages in four sentinel rooms. These had received the intervention (treated nets, house spraying or no intervention) which had been assigned to the village concerned. Window exit trap and pyrethrum spray collections were carried out in these rooms, the mosquitoes were identified, classified by abdominal condition and blood meals were squashed on to filter paper for later identification by ELISA (Service *et al.* 1986).

Malariology

Incidence of re-infection with malaria was monitored once before the interventions and four times after them (once in each quarter of 1996) by taking weekly blood slides from cohorts of 60 children aged 1–5 years per village (different children were in the cohorts for each of the five studies). The children were given three daily doses of 1.25 mg chlorproguanil and 2.5 mg dapsone per kg body weight (Watkins *et al.* 1988; Trigg *et al.* 1997) to clear pre-existing parasitaemia. Once a child became positive again for malaria parasites, he or she was treated and not requested to attend for further sampling, but those who remained free of malaria infection continued to be monitored for 7–8 weeks. Any children who were reported to have stayed away from home or who missed their blood check for more than one week were dropped from the cohort as they may have acquired malaria under different conditions from those in their homes. For graphical purposes results were calculated for each week as the number still free of malaria divided by the initial size of the cohort minus those dropped because of travel or missing sampling for two or more weeks. For statistical analysis, results from each village were calculated as number becoming positive divided by the total number of child weeks at risk and the quotients were submitted to analysis of variance by treatment and season, with results from each village treated as replications. Week 1 after treatment was omitted from these calculations because very few children became patent for infection between weeks 1 and 2 (Figure 2), presumably because of residual effects of the drugs, despite their known short half lives (Watkins *et al.* 1988).

Cross-sectional surveys were carried out monthly from the latter part of 1995 until the end of 1996 in each village on samples of children between their first and sixth birthdays. A sample of 50 such children was randomly selected by computer from the census list for each village, after excluding children who had been so selected in the previous month and, if found with parasitaemia $>$ 4000/μl, would have been treated by us. Children in the chlorproguanil-dapsone treatment cohorts were also excluded. Village health workers contacted the parents of the selected children and requested their attendance for malaria surveillance. On the surveillance day, as many as possible (generally about 35) of the list of 50 were examined. Date of birth, axillary temperature, weight, a blood slide and a blood sample for haemoglobin measurement in a Hemocue machine were taken for each child.

A system of passive surveillance was set up in the villages near Hale based on, but extending, the Pangani Falls Redevelopment Project primary health care system. A malaria assistant was appointed for each of the component hamlets of the six villages (29 hamlets in all). The assistants were given a week's training and they were kept supplied with slides, lancets, a clinical thermometer, a stock of chloroquine and record books. Meetings were held in each hamlet to explain the assistants' role. Patients who considered that they had fever were encouraged to visit their local assistant who took their axillary temperature and a blood slide, recorded their names and ages and provided chloroquine presumptively. Once a week the assistants were visited to collect their record books and slides for reading at the field station and to return the previous week's results.

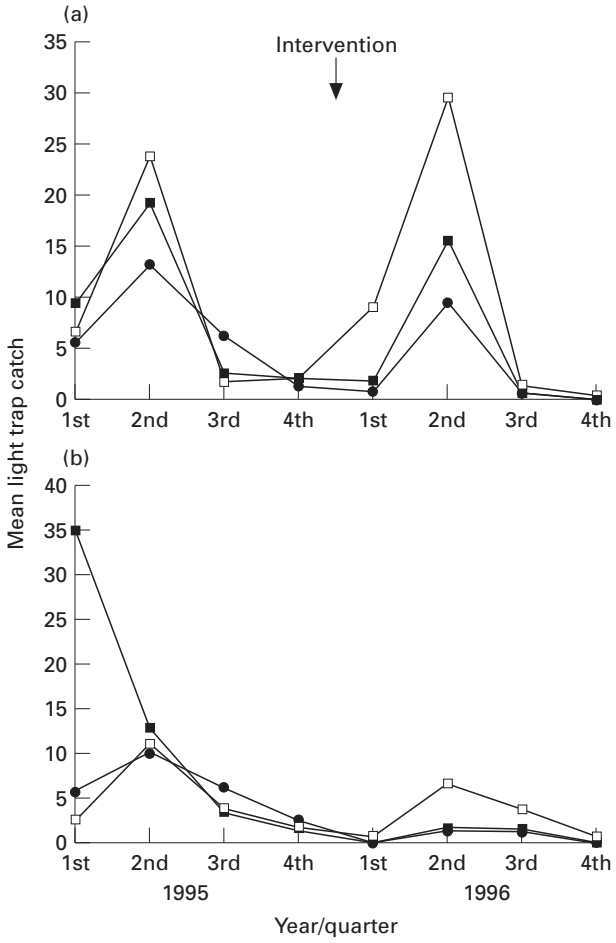

Figure 1 Mean light trap catch in each quarter of (a) *Anopheles gambiae s.l.* and (b) *An.funestus*. Data based on 36–60 trappings per group of villages per quarter. □ control; ■ nets; ● spray.

Spraying and net treatment

Spraying with microencapsulated lambdacyhalothrin (10% 'Icon CS') was carried out by teams of 10 residents from each of the four villages designated for spraying, equipped with Hudson Xpert sprayers. The teams had been given two days training and worked in pairs under a supervisor from our research team. The target dose was 30 mg a.i./m^2. This was approached by diluting 50 ml of the concentrate in a full pump charge of 8 l. It was found that on average spraying 15 houses consumed 1 litre of concentrate. The mean interior wall and roof area per house was found to be 232 m^2 which implies that the actual dose applied was about 31 mg a.i./m^2 (i.e. very close to the target). Re-spraying of the four villages was carried out in July–August 1996, i.e. 7–8 months after the initial spray.

Nets were of 100 denier knitted polyester from the Siam Dutch Co. and were available in widths of 0.7, 1.0, 1.3 and 1.8 m. After a survey of all beds and sleeping mats the necessary nets were supplied to each house, the percentages of the four sizes needed being about 5%, 45%, 45% and 5%, respectively. The mean number of nets per household and the mean number of people per net were 2.71 and 1.43. Preliminary trials showed that mean uptake of liquid per square metre after dipping and wringing was 40 ml. The target dose of lambdacyhalothrin in two of the villages was 10 mg a.i./m^2 based on the data of Curtis *et al.* (1996). However, after a few poor bioassay results it was decided to raise this to 20 mg/m^2 for the other two villages. For these two target doses, 25 or 50 ml were diluted in 10 l. Treatment was by dipping and wringing at a central point in the village, after which householders took their nets home for drying. In practice it was found that 10 l of mixture treated only 15 nets of average area 11.7 m^2, implying 42% over-dosing and/or loss from dripping after hasty wringing.

The research team visited each house the day after giving the nets to check that they had been correctly hung. No request was made to wash or not wash nets until, after

7 months net use, washing was requested and retreatment was then carried out.

Bioassays

To monitor the spray deposits, a small hut was sprayed in an unsprayed village and bioassays were carried out on the mud walls and the palm thatch door. *An. gambiae* were collected fully fed in the same village to minimize damage due to transport and exposed to the sprayed surfaces for 10 min in a WHO bioassay cone. They were then held in paper cups for 24 h with glucose solution on cotton wool. Controls were exposed for 10 min to an unsprayed mud wall and then held for 24 h.

Some bioassays of treated nets were carried out with 3 min exposure and 24 h holding (WHO 1989). However, after difficulties with control mortality, we adopted the method of observing the time for knockdown (WHO 1996) of each of a sample of 11 *An. gambiae* collected blood-fed in an untreated village. Nets were taken for testing to this village after being borrowed from the householders in the netted villages. The householders were asked how many times the nets had been washed. Part of the net (top or sides) was wrapped round a frame made of two intersecting circles of wire, of diameter about 15 cm. The mosquitoes were introduced and the time when each was first knocked down was noted, after which it was gently sucked into a mouth aspirator held inside the wire frame to avoid confusion with mosquitoes recovering and subsequently being knocked down again. Data on the knockdown time of the median mosquito of each sample are presented below. For controls, it was only required to check that with untreated netting all mosquitoes remained active for 15 min which exceeds the longest knockdown time with a treated net.

Results and discussion

Bioassays

Table 1 shows continued high insecticidal effectiveness of the spray deposits up to the time of re-spraying, when these bioassays were discontinued.

A questionnaire, supported by checking marks made with washable ink, indicated that 70% of people had chosen not to wash their nets until requested to do so just before retreatment was scheduled.

Table 2 shows that after the first treatment there was a significant effect of washing nets on time for knockdown of mosquitoes walking on them. However, in nets washed after re-treatment the effect of washing was not significant. There was an apparent, but not statistically significant, difference in the knockdown effect of the two alternative doses applied. Sides of nets, which were presumably frequently handled, tended to show reduced knockdown activity compared with the tops of the nets. After re-treatment, the time for knockdown was greatly reduced. These results are consistent with those of Curtis *et al.* (1996) from bioassays with a fixed 3-minute exposure time plus 24 h holding, but the time-for-knockdown method was found to be more sensitive than the 3 minute method which tends to give 100% mortality with nets with widely varying insecticidal power.

Table 1 Bioassays on sprayed (target dose 30 mg lambda-cyhalothrin/m^2) mud wall and palm thatch – 10 min exposure in WHO bioassay cone followed by 24-h holding period

Months after spraying	% Mortality at 24 h (no. tested)		
	Control	Mud	Thatch
0–1	7.1 (28)	100 (10)	93.3 (30)
1–2	3.3 (30)	100 (25)	100 (10)
2–3	3.2 (31)	80 (10)	93.3 (30)
3–4	2.0 (50)	100 (55)	96.4 (55)
6–9	5.0 (40)	100 (54)	100 (55)

Table 2 Median time (secs) for knockdown of samples of about 11 An. gambiae confined inside lambdacyhalothrin-treated netting which had been in domestic use (no. nets tested)

	Months after original treatment		Months after re-treatment with 20 mg/m^2	
	4–6	7–8	1–4	8
Original dose 10 mg/m^2				
unwashed:				
sides	565 (7)	—	284 (5)	—
top	—	—	213 (5)	—
washed:				
sides	728 (7)	—	308 (5)	—
top	—	—	304 (5)	—
Original dose 20 mg/m^2:				
unwashed				
sides	502 (8)	487 (5)	295 (5)	—
top	—	362 (5)	279 (5)	—
washed				
sides	643 (8)	728 (7)	310 (5)^w	322 (9)^w
top	—	598 (7)	232 (5)^w	—
F from ANOVA:				
bet. orig. doses	1.8 *n.s.*	—	0.01 *n.s.*	—
bet. wash/unwashed	7.4**	23.1**	0.85 *n.s.*	—
bet. sides/top	—	6.8*	3.6 *n.s.*	—
vs. preceding test	—	1.7 *n.s.*	144***	1.2 *n.s.*

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; w = washed after re-treatment.

Village-wide and in-house effects on vector mosquitoes

Figure 1a and b show major seasonal fluctuations in the populations of *An. gambiae s.l.* and *An. funestus* with similar catches in the pre-intervention year in the villages later assigned to control and the interventions (except for a remarkably high catch of *An. funestus* in the first quarter in one of the groups of villages). From samples in the months of January and May in the Hale villages about 5% of the *An. gambiae* complex were identified by PCR as *An. arabiensis*, the remainder being *An. gambiae s.s.* (R. H. Hunt and L. Matthews, unpublished observations). After both interventions there were marked reductions in the vector populations. Analysis of variance of the light trap catches (transformed to $\log x + 1$) was carried out treating the data from individual villages as replications (individual village

Table 3 Summaries of data (a) from Figure 1 and ELISA tests on human biting by infective *An. gambiae* and *An. funestus* in untreated or treated villages, (b) from Table 4 on human blood fed anophelines in untreated or treated rooms and (c) from Figure 2 on incidence of malaria infection in children cleared of their pre-existing infections with chlorproguanil-dapsone

	Control	Nets	Spray
(a)			
Mean catch per light trap night over all seasons:			
1995 (pre-intervention)	13.37[a]	19.12[a]	12.65[a]
1996 (post-intervention)	13.15[a]	5.38[b]	3.72[b]
Estimated no. bites/unprotected person/night (light trap catch × 1.5) (Lines *et al.* 1991)			
1995	20.06	28.68	18.97
1996	19.72	8.07	5.58
% reduction due to treatments	—	59.1%	71.7%
Sporozoite rate (no. tested by ELISA)			
1995	|– – – – – – – – – – 5.18% (1620) – – – – – – – – – –|		
1996	3.92% (1120)[a]	0.99% (303)[b]	1.02% (295)[b]
Entomological inoculation rate (sporozoite positive bites/person/night)			
1995	1.04	1.48	0.98
1996	0.773	0.080	0.057
% reduction due to treatment	—	89.6%	92.6%
(b)			
No. fed anophelines indoors + exit trap per house collection in 1996 (no. collections)			
Fed anophelines/collection	3.6 (144)[a]	0.62 (144)[b]	0.95 (144)[b]
Human blood index	81.1% (249)[a]	|– – – – 86.7% (30)[a] – – – – – – –|	
Bites on humans/night	2.9	0.54	0.82
% reduction due to treatments	—	81%	71%
Sporozoite infective bites on humans/night	0.114	0.0053	0.0084
% reduction due to treatments	—	95.3%	92.6%
(c)			
Incidence of infection per child/week (nos. in parentheses indicate no. of village cohorts each of about 60 children)			
Apr.-May 1995 (pre-intervention)	0.421(4)	0.413(4)	0.477(5)
Feb.-Mar 1996	0.247(3)	0.160(3)	0.127(3)
Apr.-May 1996	0.442(1)	0.091(1)	0.239(1)
July-Aug 1996	0.182(2)	0.056(2)	0.088(2)
Nov.-Dec. 1996	0.270(2)	0.054(2)	0.061(2)
Weighted mean post-intervention	0.264[a]	0.100[b]	0.122[b]
Mean percentage reductions due to treatments	—	62.1%	53.7%

Figures in the same row which share a superscript letter do not differ at the 5% level of significance.

Table 4 Total no. anophelines collected by pyrethrum spray indoors and window exit trap in four rooms in each of the 12 villages once a month from March to November 1996

	Controls	Nets	Spray
Fed, indoors	296	43	60
Fed, exit	223	47	77
Unfed, indoors	26	16	97
Unfed, exit	238	102	100
Total	783	208	334
% fed	66.2%	43.3%	41.0%
% indoors	41.1%	28.4%	47.0%
No. collections	144	144	144

data not shown in Figure 1). The analysis showed highly significant impacts of the interventions (year–treatment interactions gave $F_{126}^{2} = 7.63$, $P < 0.001$). However, the effects of the nets or spraying did not differ significantly ($F = 0.029$, *n.s.*), nor was there a significant difference in impact on *An. gambiae s.l.* or *An. funestus*, ($F = 0.03$, *n.s.*), nor in the percentage impact in different quarters of the year ($F = 0.66$, *n.s.*). Table 3a converts the light trap catches to estimates of the biting per unprotected person per night using the relationship found by Lines *et al.* (1991).

Most of the mosquito specimens for ELISA testing for circumsporozoite protein came from the second quarters (wet season) of each year. There were too few from other seasons to check convincingly for seasonal variations in sporozoite rate and the data were pooled over seasons. Also, in the preintervention year, labelling was sometimes inadequate to separate all the villages and the data are presented pooled over all three groups of villages. The pooled data showed highly significant reductions due to each intervention in the circumsporozoite protein rate, presumably due principally to shortening of the mean mosquito lifespan by the insecticide deposits (Magesa *et al.* 1991). Multiplying the biting by the circumsporozoite rates indicated reductions in the entomological inoculation rates on unprotected people in the villages by about 90% as a result of nets or spraying.

Table 5 Cross sectional surveys of children between their 1st and 6th birthdays for parasitaemia, fever and the combination of fever with parasitaemia

Quarters & year	Contr./ nets/ spray	*n*	Malaria parasites				Fever ≥ 37.4 °	% reported fever and/or ≥ 37.4 °	Combination % rep. fever and/or > 37.4 ° with ≥ 4000/μl
			% + ve	% > 4000 /μl	% > 16000/μl	g.m(/μl)			
3rd & 4th q.'95	C	104	94.2^{a}	41.3^{a}	8.7^{a}	6078^{a}	21.2^{a}	30.8^{a}	14.4^{a}
(Pre-intervention)	N	93	95.7^{a}	34.4^{ab}	6.5^{a}	5114^{ab}	21.5^{a}	30.2^{a}	12.9^{a}
	S	86	88.4^{a}	24.4^{b}	9.3^{a}	4557^{b}	24.4^{a}	34.9^{a}	8.1^{a}
1st q.'96	C	259	83.4^{a}	27.0^{a}	5.8^{a}	3958^{a}	23.9^{a}	27.8^{a}	8.9^{a}
	N	227	84.1^{a}	26.0^{a}	5.3^{a}	4457^{a}	17.2^{a}	22.9^{a}	8.4^{a}
	S	164	76.2^{a}	25.6^{a}	2.4^{a}	2881^{a}	17.7^{a}	21.3^{a}	7.9^{a}
2nd q.'96	C	339	85.3^{a}	28.3^{a}	4.1^{a}	3726^{a}	21.2^{a}	26.0^{a}	7.7^{a}
	N	322	79.2^{b}	20.8^{b}	2.2^{a}	2485^{b}	22.0^{a}	26.4^{a}	5.3^{a}
	S	291	80.1^{ab}	25.8^{ab}	1.7^{a}	2614^{b}	19.6^{a}	24.1^{a}	7.9^{a}
3rd q.'96	C	300	81.7^{a}	30.3^{a}	5.0^{a}	3890^{a}	22.0^{a}	28.7^{a}	10.0^{a}
	N	320	73.4^{b}	17.8^{B}	1.6^{b}	2175^{B}	16.3^{ab}	20.3^{b}	5.9^{a}
	S	282	73.0^{b}	20.9^{b}	2.1^{ab}	2524^{B}	13.8^{b}	18.1^{B}	6.0^{a}
4th q.'96	C	240	67.5^{a}	26.3^{a}	2.1^{a}	2773^{a}	17.5^{a}	21.7^{a}	9.2^{a}
	N	237	60.3^{b}	15.6^{B}	1.7^{a}	1907^{B}	18.6^{a}	20.7^{a}	4.2^{b}
	S	212	63.7^{ab}	19.3^{ab}	0.9^{a}	1854^{B}	15.1^{a}	18.9^{a}	6.1^{ab}
Observation total no:									289
If no association, exp. no:									244.8

g.m, geometric mean parasite density.
Within each column and quarter of the year figures sharing the same superscript letter do not differ statistically significantly: lower case letters are used for the 5% level and a capital letter where there is difference from the control at the 1% level.

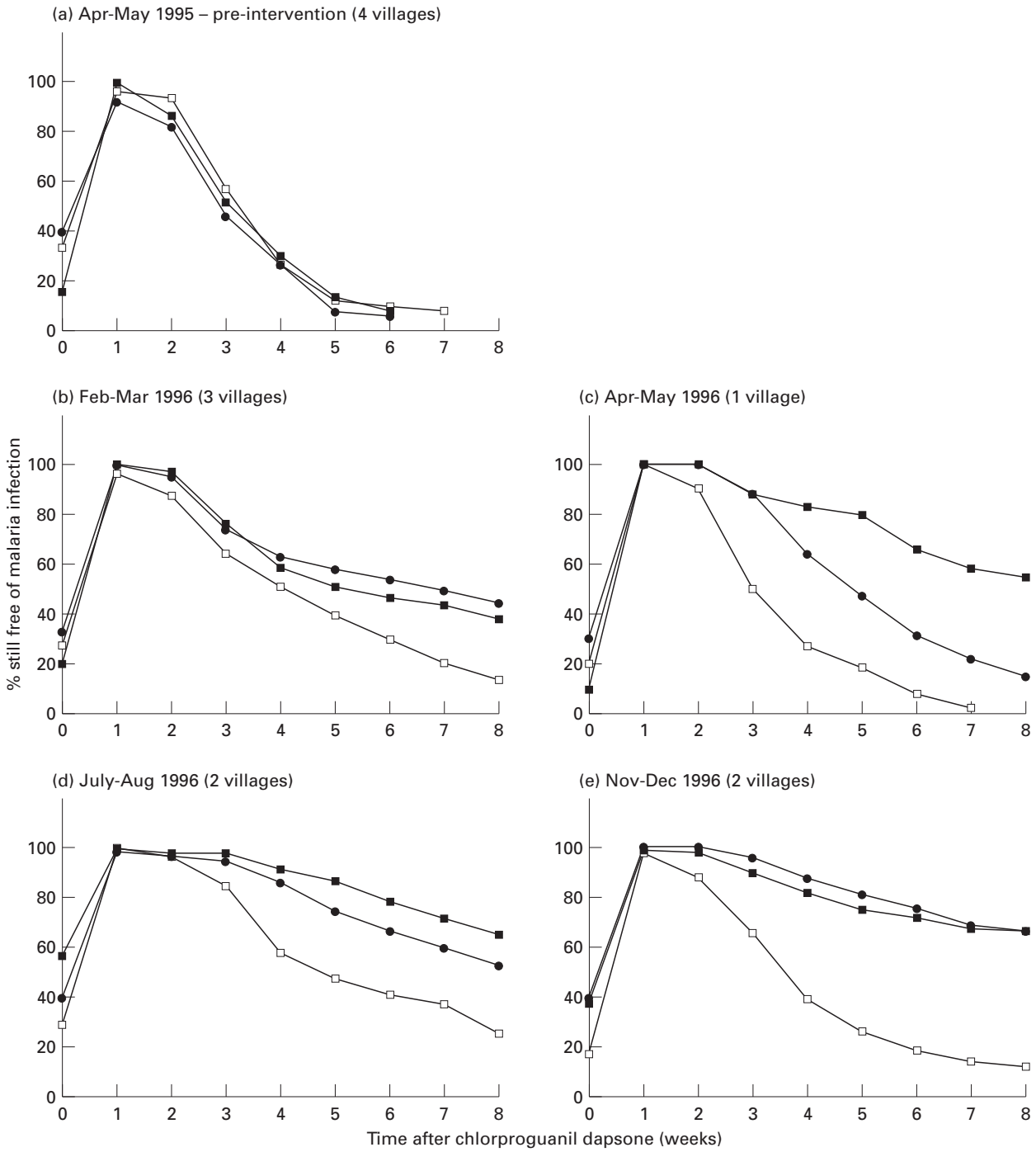

Figure 2 Percentage of cohorts of 60 children per village still free of malaria infection at successive weeks after treatment with chlorproguanil dapsone. See Methods section for details of methods of calculation. □ control; ■ nets; ● spray.

Table 4 indicates reductions in the number of bloodfed mosquitoes and in the proportion remaining indoors in rooms provided with treated nets. Analysis of variance indicated insignificant differences in numbers of bloodfeds between the nets and spraying ($F = 0.87$). However, comparison of data in sections (a) and (b) of Table 3

suggested that, whereas the effect in the sprayed rooms could be entirely explained by the reduction in the village mosquito populations (as indicated by the light traps), 35% fewer blood fed mosquitoes were collected in netted than in sprayed rooms which suggests an additional personal protection effect in the rooms provided with nets. However, this difference was not statistically significant. Blood meal identification (S.Tho, unpublished observations) showed no evidence for any diversion from predominant anthropophily as a result of the interventions.

Incidence of malaria infection

Figure 2 and Table 3c show the results of five experiments in which prior infection was cleared with chlorproguanil-dapsone and the rate of re-infection was observed. The graph for April–May 1995 was obtained by re-arranging the data presented by Trigg *et al.* (1997). These data show no significant difference in incidence of positivity for infection per child week at risk between the villages later assigned to controls or the two interventions ($F^2_8 = 0.47$, from analysis of variance, treating as replications data from individual villages (individual village data not shown in Table 3)). Each of the trials in 1996 showed reductions in malaria incidence as a result of each intervention. Two way analysis of variance between all the treated and the control villages showed a highly significant difference between these categories ($F^2_{12} = 18.8$), a significant difference between the incidence rates in the four seasons ($F^3_{12} = 5.2$) but no significant difference in the effect of the treatments at different seasons (interaction $F^6_{12} = 2.14$). Restricting the analysis to the two treatments showed no significant difference between the results for nets and spraying ($F^1_8 = 0.86$). Table 3c shows that the percentage reductions in incidence of re-infection (54–62%) were not as great as would have been expected from the reductions in the entomological inoculation rate (90–95% ; Table 3a). In the control villages, the malaria incidence varied with season (0.44 in April–May *vs*. 0.18 in July–August). However, the seasonal difference was not as great as would have been expected from the entomological results (Figure 1).

One possible explanation of these discrepancies between the percentage reductions achieved by the treatments as measured by the incidence and the entomological inoculation rate is that some of the recurrences of parasitaemia were due

Table 6 Cross sectional surveys of haemoglobin concentrations in children between their 1st and 6th birthdays and the association of haemoglobin level with parasitaemia

		Mean Hb (g/dl)			
			With parasitaemia		
Quarter & year	Contr. /nets /spray	Whole sample (n)	≤ 4000/μl (n)	≥ 4000/μl (n)	% with Hb < 8 g/dl
3rd & 4th q.'95	C	8.69^{a} (104)	9.08^{a} (63)	8.12^{a} (41)	35.6^{a}
(Pre– intervention)	N	9.25^{b} (93)	9.76^{b} (61)	8.29^{a} (32)	19.4^{b}
	S	9.04^{ab} (86)	9.34^{ab} (65)	8.16^{a} (21)	32.6^{a}
1st q.'96	C	9.44^{a} (259)	9.71^{a} (187)	8.70^{a} (69)	23.6^{a}
	N	9.95^{B} (227)	10.20^{b} (168)	9.25^{a} (59)	13.7^{B}
	S	9.76^{ab} (164)	10.01^{ab} (122)	9.06^{a} (42)	15.2^{b}
2nd q.'96	C	9.55^{a} (313)	9.69^{a} (234)	9.12^{a} (79)	12.4^{a}
	N	9.74^{ab}(352)	9.86^{ab} (202)	9.45^{b} (84)	10.2^{a}
	S	9.96^{b} (258)	10.10^{b} (202)	9.45^{b} (75)	9.7^{a}
3rd q.'96	C	9.24^{A} (297)	9.47^{a} (208)	8.69^{a} (89)	21.0^{a}
	N	10.08^{b} (320)	10.21^{B} (263)	9.44^{b} (57)	8.1^{B}
	S	9.82^{c} (282)	10.02^{B} (223)	9.07^{ab} (59)	10.6^{B}
4th q.'96	C	9.39^{a} (240)	9.56^{a} (177)	8.89^{a} (63)	17.5^{a}
	N	10.18^{B} (237)	10.26^{B} (200)	9.72^{b} (37)	8.9^{B}
	S	10.24^{B} (212)	10.42^{B} (171)	9.49^{ab} (41)	7.5^{B}
2nd q.'97	C	9.01^{a} (268)	9.10^{a} (220)	8.59^{a} (48)	24.1^{a}
	N	10.10^{B} (395)	10.23^{B} (342)	9.24^{B} (53)	6.5^{B}

Within each column and quarter of the year, figures sharing the same superscript letter do not differ statistically significantly: lower case letters are used for the 5% level and a capital letter where there is difference from the control at the 1% level.

Table 7 Cross-sectional surveys of prevalence of children between their 1st and 6th birthdays with weight for age z-scores less than −2

Quarters and year	Contr./ Nets/Spray	*n*	Age adjusted % WAZ < −2	95% confidence limits
4th q.'95	C	40	31.8[a]	± 11.4
	N	50	25.7[a]	± 13.5
	S	44	26.5[a]	± 11.6
1st q.'96	C	117	29.0[a]	± 7.9
	N	76	20.6[a]	± 9.1
	S	83	22.9[a]	± 8.9
2nd q.'96	C	170	30.6[a]	± 6.9
	N	125	23.2[ab]	± 7.4
	S	123	20.0[b]	± 6.9
3rd q.'96	C	149	31.8[a]	± 7.4
	N	158	18.0[B]	± 5.8
	S	153	17.4[B]	± 5.9
4th q.'96	C	168	22.0[a]	± 6.9
	N	225	20.3[a]	± 5.8
	S	161	20.3[a]	± 7.0
2nd q.'97	C	344	46.8[a]	± 7.9
	N	442	46.9[a]	± 6.3

Within each quarter figures not sharing the same superscript letter differ statistically significantly: lower case letters are used for the 5% level and capital letters where there is a difference from control at the 1% level.

to recrudescence of infections which, though not patent at week 1 after treatment, had not in fact been eliminated. However, PCR of polymorphic gene loci suggested that infections which appeared at week 3 were genetically different from those present before drug treatment (Trigg *et al.* 1995). Recently more convincing evidence against the recrudescence hypothesis has been provided by taking a group of children to spend a few weeks at an altitude too high for there to be appreciable risk of infective mosquito bites (C.A.Maxwell *et al.* unpublished observation).

The average rate of incidence of re-infection was 0.264 per child week in the control villages in 1996. This is only 4.8% of the corresponding entomological inoculation rate calculated on a weekly basis (7×0.773 from Table 3a). This estimate of 4.8% for the parameter b in the Macdonald equation is of the same order of magnitude as that found by Pull & Grab (1974) but is much less than that calculated by Pringle & Avery-Jones (1966). Our data lead to an incidence calculated on a monthly basis of $4 \times 0.264 = 1.05$, i.e. several times higher than the highest rate reported by Snow *et al.* (1997) from a holoendemic area near Lake Victoria. The discrepancy may be because transmission is actually more intense in our area and/or because the infants studied by Snow *et al.* were probably partially protected from biting by diversion of most of the mosquitoes to their mothers sleeping with them. The somewhat older children in our samples generally sleep independently and therefore are presumably not subject to so much diversion of mosquito biting.

Cross-sectional surveys for malaria morbidity

The control villages in Table 5 (col.4) show that malaria parasitaemia declined spontaneously during the trial. Vector densities were low in the very dry weather at the end of 1996 (Figure 1) but the decline in parasitaemia was apparent earlier in 1996 when vector densities in the control villages were still high. The provision of a supply of chloroquine to health assistants in the villages in the Hale area may have contributed to this, as may the apparently 20% lower sporozoite rate (Table 3) in the 1996 controls than in 1995, though the difference was not statistically significant ($\chi_1^2 = 2.1$).

Comparing the controls with the two interventions within each quarter of the year, the netted villages did not differ significantly (χ^2 or *t*-tests) from the controls for any of the measures of parasitaemia (Table 5, cols. 4–7) in the pre-intervention period but, by the 3rd and 4th quarters of 1996, they were highly significantly less than controls in percentage with more than 4000 parasites/μl (col.5) and in geometric mean parasite density (col.7). For the sprayed villages the evidence for this improvement was less convincing since they differed significantly from controls for these two parameters in the pre-intervention period. However, the difference in mean parasite density (col.7) became much more highly significant in the last two quarters of the experiment.

18–35% of children had axillary temperatures above 37.4° or recent fever reported by their mothers (Table 5, col.8) but fewer than 1% in these cross-sectional surveys had temperatures above 38.9% (data not shown). Prevalence of reported or measured fever, or fever combined with moderately high parasitaemia, did not differ between the three categories of village until the third and fourth quarters of 1996 when some evidence appeared for significant benefit of each treatment, compared with the controls.

In no case was there a significant difference between the two treatments in any quarter of the year or for any parameter.

The overall number of cases of fever combined with parasitaemia > 4000/μl were slightly but significantly more than expected on the null hypothesis of no association between these parameters ($\chi^2 = 32$). Such an association has previously been found in this area by Lyimo *et al.* (1991).

Haemoglobin concentrations were consistently lower in those with high malaria parasitaemia on the day of sampling (Table 6, cols. 4 and 5), than in those with low or zero parasitaemia.

Table 8 Passive surveillance in 6 villages via resident health workers. Pre-intervention (3rd & 4th quarter 1995) and postintervention (1996)

Quarters & year	Control /nets /spray	*n*	Malaria parasites (%)			Axillary temperature (%)		Parasites with fever (%)	
			+ ve	≥ 4000/μl	≥ 16000/μl	> 37.4°	> 38.9°	> 37.4°with ≥ 4000/μl	> 38.9°with ≥ 16000/μl
Children < 6 years									
3rd & 4th '95	C	142	68.0[a]	32.0[a]	17.3[a]	59.2[a]	16.2[a]	21.1[a]	5.6[a]
	N	125	70.7[a]	32.3[a]	19.0[a]	58.1[a]	23.2[a]	15.2[a]	3.2[a]
	S	218	69.2[a]	31.2[a]	16.7[a]	51.1[a]	17.8[a]	17.0[a]	5.5[a]
1st & 2nd '96	C	257	73.3[a]	29.3[a]	11.1[a]	54.0[a]	16.8[a]	18.7[a]	4.7[a]
	N	224	65.2[ab]	22.3[ab]	7.6[a]	52.8[a]	22.1[a]	16.1[a]	2.2[a]
	S	252	58.7[b]	18.2[b]	11.9[a]	55.8[a]	9.0[b]	14.7[a]	4.0[a]
3rd & 4th '96	C	214	54.2[a]	16.3[a]	4.2[a]	49.5[a]	20.9[a]	8.4[a]	0.[a]
	N	181	60.0[a]	17.8[a]	6.6[a]	55.7[ab]	18.9[a]	10.5[a]	5.0[a]
	S	161	49.7[a]	15.5[a]	5.6[a]	61.4[b]	17.2[a]	7.4[a]	4.3[a]
Observed total nos.								256	67
If no association, exp. no.								228.0	32.9
Children > 6 years									
3rd & 4th '95	C	358	50.7[a]	9.9[a]	3.1[a]	70.1[a]	14.5[a]	5.9[a]	1.1[a]
	N	232	43.9[b]	9.3[a]	5.1[a]	51.1[b]	13.0[a]	2.1[a]	0.4[a]
	S	577	42.6[b]	11.6[a]	3.6[a]	50.8[b]	14.6[a]	5.4[a]	0.5[a]
1st & 2nd '96	C	503	40.6[a]	8.9[a]	3.3[a]	44.3[a]	8.7[a]	4.7[a]	0.8[a]
	N	501	45.5[a]	7.0[a]	1.0[b]	40.6[a]	9.4[a]	3.8[a]	0[a]
	S	527	39.6[a]	7.6[a]	2.6[a]	53.6[b]	7.6[a]	3.8[a]	0.2[a]
3rd & 4th '96	C	504	35.2[a]	5.1[a]	1.0[a]	39.8[a]	9.8[a]	3.0[a]	0.4[a]
	N	392	30.1[a]	4.1[a]	1.8[a]	39.6[a]	9.0[a]	3.8[a]	0.5[a]
	S	457	34.1[a]	6.3[a]	2.2[a]	53.6[b]	12.1[a]	3.5[a]	0.4[a]
Observed total nos.								166	19
If no association, exp. nos.								157.3	11.4

Within a column and pair of quarters, figures which share the same superscript letter do not differ at the 5% level of significance.

The villages assigned for spraying showed no significant difference (by t or χ^2 test) from control levels of haemoglobin (Table 6, col.3) or percentage with anaemia (which we define as haemoglobin < 8 g /dl, col.6) in the pre-intervention period, but these differences had become highly significant by the last two quarters of 1996. The netted villages also showed highly significantly greater mean haemoglobin concentrations and lower prevalence of anaemia than the controls in the last two quarters of 1996. Part of this difference may be due to an inherent difference between these villages, as indicated by just-significant differences between them in the pre-intervention period. However, we are confident that prolonged protection of children under nets did have a beneficial effect in reducing anaemia, as only in the 3rd and 4th quarters of 1996 did mean haemoglobin rise to 10.0 g/dl and anaemia drop to less than 10%. This beneficial effect was confirmed (Table 6, last 2 rows) in a further large survey in 1997 in the netted villages and in a new set of control villages (the original control villages having by then been given nets). A similar beneficial effect of prolonged use of treated nets in coastal Tanzania has been reported by Shiff *et al.* (1996).

During the cross-sectional surveys children were weighed. Weight-for-age z-scores were calculated using Epinut, part of the Epi-info suite of programmes. A considerable number of the children had to be excluded because of imprecise age data.

Table 7 shows age-standardized prevalences of underweight children (i.e. those with a z-score < −2) in each quarter of the year and in control, netted and sprayed villages. The significance of differences between the interventions and controls within each quarter were assessed using age stratum weighted Mantel Haenszel χ^2 tests. The observed proportions with low weight-for-age were higher in each quarter in the control villages, but this difference was not significant in 1995, before the interventions started, nor in

Table 9 Costs in US$ of providing for one year treated nets or house spraying for 1000 people in 258 houses which would require 700 nets:

	Treatment	
	1/yr	2/yr
Treated nets		
Annual replacement cost of nets (700 nets @ $4 each with life of 4 years)	700	700
Cost of insecticide (2.33 l at $120*/litre: 50 ml of 10% Icon CS treat 15 nets)	280	560
Labour cost (6 person days for treatment and house checking @ $4/person day)	24	48
Transport of personnel	20	40
Total	1024	1348
House spraying		
Cost of insecticide for 258 houses (1 litre of 10% Icon CS treats 15 houses)	2064	4128
Labour cost (50 person days)	200	400
Transport of personnel:	40	80
Total	2304	4608

* price in USA

the first quarter of 1996. In the second quarter the differences were on the borderline of significance and, in the third quarter, both the netted and sprayed villages had highly significantly fewer underweight children than in the controls. However, in the fourth quarter of 1996, the difference disappeared and was not seen in the further sample taken in 1997. It seems possible that the very high prevalence of underweight at that time was due to malnutrition following the very dry weather at the end of 1996 already mentioned.

Other authors have reported an association of anthropometric indicators with malarial morbidity (Mbago & Namfua 1992). Shiff *et al.* (1996) reported an association with transmission and asymptomatic parasitaemia and they found an improvement in weight gain of children between 1 and 3 years in villages with impregnated bednets compared with controls. In our case any such effect was not sustained.

Passive surveillance by resident health assistants

Table 8 shows a similar downward trend in 1995–6 in malaria parameters found by passive surveillance of those reporting sick to health assistants, as was seen in cross-sectional surveys (Table 5). However, Table 8, col.6, shows that the passive surveillance detected far more children with very high parasitaemia (the slide reading was done by the same microscopists for both forms of surveillance). The prevalence of very high fever ($> 38.9\,^{\circ}C$) was also much higher from passive surveillance (Table 8, col.8) than the less than 1% found in cross sectional surveys. This trend might be expected among those considering themselves to be sick, but it may have been exaggerated by errors in using clinical thermometers by the health assistants who only received minimal training. There was, however, a clear association among children and adults between very high fever and very high parasitaemia (Table 8, bottom of col.10).

In almost no cases did the passive surveillance give significant evidence for reductions in malaria or associated fever as a result of either intervention against the vectors. It is not clear why this was so but it is a warning not to expect to see dramatic effects on malaria morbidity as recorded by village health workers when vector control measures are introduced into holoendemic areas.

Cost and acceptability

Table 9 shows estimates of the costs of provision of protection for 1000 people per year by treated nets or spraying based on observed consumption of insecticide, reported cost of nets and 10% Icon CS insecticide, labour costs in Tanzania and an estimated 4 years durability of nets. In certain parts of the study villages rats, apparently seeking the kapok stuffing of mattresses, gnawed nets and seriously reduced their durability. Not included in Table 9 are the costs of the spray pumps and the protective clothing needed by the

spray teams, since these items are durable. The calculations are presented for two annual treatments as in this trial, or one treatment which Tables 1 and 2 suggest would be enough. A net treatment programme would be less costly than house spraying. Some people believe that tropical villagers should be made to pay for bednets and net treatment. However, in countries where there has been a house spraying programme this has always been provided by the health service (as with other preventive health measures such as vaccination). Despite this inconsistency in likely methods of payment, the comparisons in Table 9 are of some relevance as someone has to pay, whichever method is adopted.

Questionnaires administered to householders indicated general satisfaction with both methods of vector control and, unlike in countries with long histories of house spraying, there was no problem of refusals of access for spraying. The employment of local villagers of both sexes as the spray teams may have helped with this, but it added to the training costs (not shown in Table 9) because a new team had to be trained for each of the villages.

Towards the end of the trial there were repeated demands from the inhabitants of the sprayed villages (as well as the control villages) for the promised nets to be delivered on time. This was the clearest indication of how welcome a gift of nets is in this area of Tanzania. Though house spraying was assented to there was no similar demand for its continuation.

Acknowledgements

We are grateful to C. Chambika, L. George, the late M. Jumanne, H. Mbwana, S. Mkongewa, J. Myamba, A. Nuru, H. Othmani, M. Sudi and M. Zuwakuu for technical assistance. ELISAs were carried out by O. Aina, L. Matthews and S.Tho, sibling species identification with PCR by R. H. Hunt and L. Matthews and computing by L. Kiaga and J. Curtis. J. K. Trigg supervised the earlier trial with chlorproguanil-dapsone in the baseline year.

Lambdacyhalothrin was provided by Zeneca and chlorproguanil-dapsone by Dr W. Watkins.

Financial support was provided by the British Overseas Development Administration and Medical Research Council and by the Pangani Falls Redevelopment Project.

Clearance for the project was provided by the Tanzanian Commission for Science and Technology and National Institute for Medical Research, the Director General of which gave permission for publication of this paper.

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