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EVALUATION OF LAMBDACYHALOTHRIN-IMPREGNATED BEDNETS IN A MALARIA ENDEMIC AREA OF INDIA. PART 3. EFFECTS ON MALARIA INCIDENCE AND CLINICAL MEASURES

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ABSTRACT. In Indian villages with high malaria endemicity use of nylon bednets treated at 25 mg/m^2 at 6-month intervals for 3 years caused significant reductions in malaria incidence, slide positivity rate, slide falciparum rate, annual parasite index, and parasite rate in the entire population, as well as reductions in rates of splenomegaly and anemia in children. In villages with untreated nets, considerable reduction also occurred in these parameters except for the rate of splenomegaly. In the village without nets, a relatively small drop occurred in the parasite rate and anemia but no change occurred in malaria incidence, and an increase occurred in the rate of splenomegaly. The trial thus showed the efficacy of impregnated bednets against malaria in the forested hills of Orissa State where the existing control strategy based on indoor residual spraying of DDT has remained incapable of interrupting malaria transmission.

KEY WORDS Bed-nets, lambdacyhalothrin, India, malaria incidence, parasite rate, splenomegaly, anemia

INTRODUCTION

Use of untreated bednets did not reduce incidence of malaria attacks in children in the Republic of Congo (Trape et al. 1987) or malaria parasite prevalence in Papua New Guinea (Burkot et al. 1990). However, routine use of bednets was associated with low parasite and splenomegaly rates in Gambian children (Bradley et al. 1986). Use of bed-nets treated with a low dose of permethrin and mass drug administration temporarily suppressed malaria parasitemia in children in East Malaysia (Hii et al. 1987). Permethrin-treated mosquito nets improved the packed cell volume and reduced clinical attacks of malaria and the rate of splenomegaly in Gambian children (Snow et al. 1988). Permethrin- and lambdacyhalothrin-treated nets caused a reduction in slide positivity for falciparum malaria in some villages but not in others (Lyimo et al. 1991) but delayed the return of malaria parasitemia in all villages after chemotherapy (Msuya and Curtis 1991) in Tanzanian children. Deltamethrin-treated nets caused significant reduction in the rate of splenomegaly in Mali (Ranque et al. 1984) and in malaria incidence in China (Lu 1991) and Assam, India (Jana-Kara et al. 1995). In Orissa State in India use of cyfluthrin-treated nets by all householders in mining settlements caused reductions in malaria incidence, slide positivity, and hospital admissions due to malaria (Yadav and Sharma 1994). The evidence for malaria control by means of pyrethroid-impregnated bednets was reviewed in the recent book by Lengeler et al. (1996). Recent studies in Africa showed that use of impregnated bednets significantly reduced child mortality (Binka et al. 1996, Nevill et al. 1996).

Because most studies have been done on children and experience on the use of lambdacyhalothrin-treated bednets, or against malaria transmitted by *Anopheles culicifacies* Giles was very limited, we conducted a longitudinal field trial on people of all ages to assess the impact of lambdacyhalothrin-impregnated nylon bednets on malaria morbidity and other clinical measures in a malaria endemic area of India. We reported earlier that the use of lambdacyhalothrin-impregnated bednets caused a significant impact on the vector population (Sampath et al. 1998b).

MATERIALS AND METHODS

Study area

Details on study area, climate, population, malaria endemicity, and distribution of bednets were discussed earlier (Sampath et al. 1998a). Briefly, the trial was conducted in forested hamlets in Kuarmunda Primary Health Centre (PHC) near the city of Rourkela in Orissa State. In May 1990, nylon nets treated with lambdacyhalothrin at 25 mg/m^2 were given to all households in 4 villages (total of 6 hamlets), untreated nets were given in 5 villages (5 hamlets), and one village (8 hamlets) without nets was used as a control. Based on bioassays and availability of supplies the nets were reimpregnated in June 1991, January 1992, June 1992, and January 1993.

Malaria incidence

One year's baseline data on malaria incidence were collected beginning in May 1989. Under the primary health care system, active and passive malaria surveillance was routinely conducted in the villages with satisfactory efficiency. This was made possible by regular visits of multipurpose health workers on assigned days supervised by the PHC staff, adequate supply of antimalarial drugs, and by

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ensuring that no staff position remained vacant. Blood smears were routinely taken from persons reporting with active fever or a history of febrile illness. Data collected by this system from May to November 1989 were taken from the PHC records. From December 1989 on surveillance was conducted by the resident surveillance workers in each village who had been specifically appointed for this trial. Each household, duly enumerated and coded, was visited on a fixed day every week. In case of active fever (>37.4°C) or a history of febrile illness between the weekly home visits, thick and thin blood smears were taken on a glass slide by aseptic finger prick. Information on house code, patient's name, age, sex, date of onset of fever, and smear code was entered on a printed surveillance form. Presumptive treatment with chloroquine (10 mg/kg) was given. Slides were brought to the parasitology laboratory of the Field Station of the Malaria Research Centre in Rourkela on the same day.

Smears were stained with Jaswant Singh Bhattacharji (JSB) stain (Singh and Bhattacharji 1944) and examined under oil immersion. Confirmed malaria cases were treated according to the standard schedule (*Plasmodium falciparum*: 25 mg/kg chloroquine including presumptive dose in 3 divided doses + 45 mg primaquine; *Plasmodium vivax*: 10 mg/kg chloroquine + 75 mg primaquine in 5 divided doses; *Plasmodium malariae*: 10 mg/kg chloroquine). Primaquine was not given to infants and pregnant women. *Plasmodium falciparum* cases not responding to chloroquine therapy were given sulphadoxine–pyrimethamine on follow up.

Malaria parameters including annual parasite index (API, malaria cases/1,000 population/year), slide positivity rate (SPR, % slides positive for malaria), and slide falciparum rate (SfR, % slides positive for *P. falciparum*) were calculated. Recrudescences or breakthroughs of parasitemia within 28 days after the first episode were not counted as new infections. All new episodes of malaria in 1 year were treated for analysis of the API. Analysis of the longitudinal data for the 3 comparison groups was done by calculating relative risk with 95% confidence intervals and the significance of differences was determined by χ^2 test.

Parasite rate

At the end of the transmission season cross-sectional surveys were conducted. Cohorts of people selected randomly were examined for the presence of malaria parasitemia in each of the 3 areas. A baseline survey was done in January 1990 and surveys were conducted in January 1991 and January 1992 during the intervention period. Parasite rates and relative risk were calculated.

Splenomegaly in children

During February 1990 (baseline) and 1993 (final), cross-sectional spleen surveys of children aged 2–9 years were done in the villages with treated nets or without nets. In the villages with untreated nets surveys were done in 1991 and 1993. Spleens were palpated according to Hackett's method (Bruce-Chwatt 1980). The rate of splenomegaly and average grade of enlarged spleen (AES) were compared. Relative risk was calculated and a χ^2 test was applied to test the significance of differences in proportions.

Hemoglobin concentration and prevalence of anemia

A cross-sectional survey was done on children aged 2–9 years in September 1991 (i.e., during the 2nd year of intervention) to measure the hemoglobin (Hb) concentration and thereby the prevalence of anemia. Another survey was done in the final year of the trial (i.e., in February 1993). Twenty microliters of free-flowing blood from a finger prick was added to 5 ml of Drabkin's reagent to measure the Hb concentration by the cyanmethemoglobin method and anemia was graded by the following criteria: severe anemia, Hb < 7 g/dl; moderate anemia, 7–10 g/dl; and mild anemia, <11 g/dl (for age 6 months to 5 years) or <12 g/dl (for age ≥ 6 years) (DeMaeyer et al. 1989). Proportions of children with various anemia grades were compared by $\chi^2_{M\text{-}H}$ test and mean Hb concentrations were compared by the *t*-test.

RESULTS

Changes in malaria incidence parameters API, SPR, and SfR in the 3 areas during the baseline and 3 years of intervention are shown in Table 1. The SPR is illustrated in Fig. 1. In villages without nets, malaria incidence increased during first intervention year but declined thereafter reaching the original level in year 3. In villages with untreated nets an increase in malaria incidence also occurred during first intervention year, but thereafter declined markedly. In contrast, a continuous decline in malaria incidence occurred in the villages with treated nets. Based on the malaria parameters given in Table 1, a concurrent analysis of relative risk (ratio of 2 proportions) of malaria in the 3 areas during the baseline and each of the 3 intervention years has been done and the data are presented in Table 2. Relative risk, as determined by API, SPR, and SfR parameters, ranged between 0.33 and 0.39 for the treated nets vs. no nets group, 0.71 and 0.84 for the treated nets vs. untreated nets group, and 0.46 and 0.50 for the untreated nets vs. no nets group. Differences in malaria incidence for the above pairs of comparison groups were significant.

Changes in the SPR by age within the treated net villages from the baseline year to the 3rd year of intervention as analyzed by a χ^2 test showed significant reduction in all the age groups (Fig. 2), except for the 15 to 19 and 20 to 29-year-old

Table 1. Changes in malaria parameters in villages with lambdacyhalothrin-treated nets, untreated nets, and no nets.[1]

Year	Population	BSE	Malaria cases					API	SPR	SfR
			Pf	*Pv*	*Pm*	*Pf* + *Pv*	Total			
Baseline year[2]										
No nets	626	303	122	13	2	3	140	224	46.2	41.2
Untreated nets	1,089	715	249	21	4	1	275	253	38.5	34.9
Treated nets	1,134	746	286	10	8	3	307	271	41.2	38.7
Intervention year 1										
No nets	786	646	279	51	7	7	344	438	53.3	44.3
Untreated nets	1,226	874	280	42	5	5	332	271	37.9	32.6
Treated nets	1,147	645	154	30	5	2	191	167	29.6	24.2
Intervention year 2										
No nets	808	521	228	31	4	3	266	329	51.1	44.3
Untreated nets	1,328	716	209	27	3	2	241	182	33.7	29.5
Treated nets	1,187	614	156	19	3	0	178	150	28.9	25.4
Intervention year 3										
No nets	814	398	153	25	5	2	185	227	46.5	38.9
Untreated nets	1,403	637	114	28	1	4	147	105	23.1	18.5
Treated nets	1,220	496	75	15	1	0	91	75	18.4	15.1

[1] BSE, blood smears examined; *Pf, Plasmodium falciparum* cases; *Pv, Plasmodium vivax* cases; *Pm, Plasmodium malariae* cases; API, annual parasite index/1,000 population; SPR, slide positivity rate (%); SfR, slide falciparum rate (%).
[2] May 1989 to April 1990.

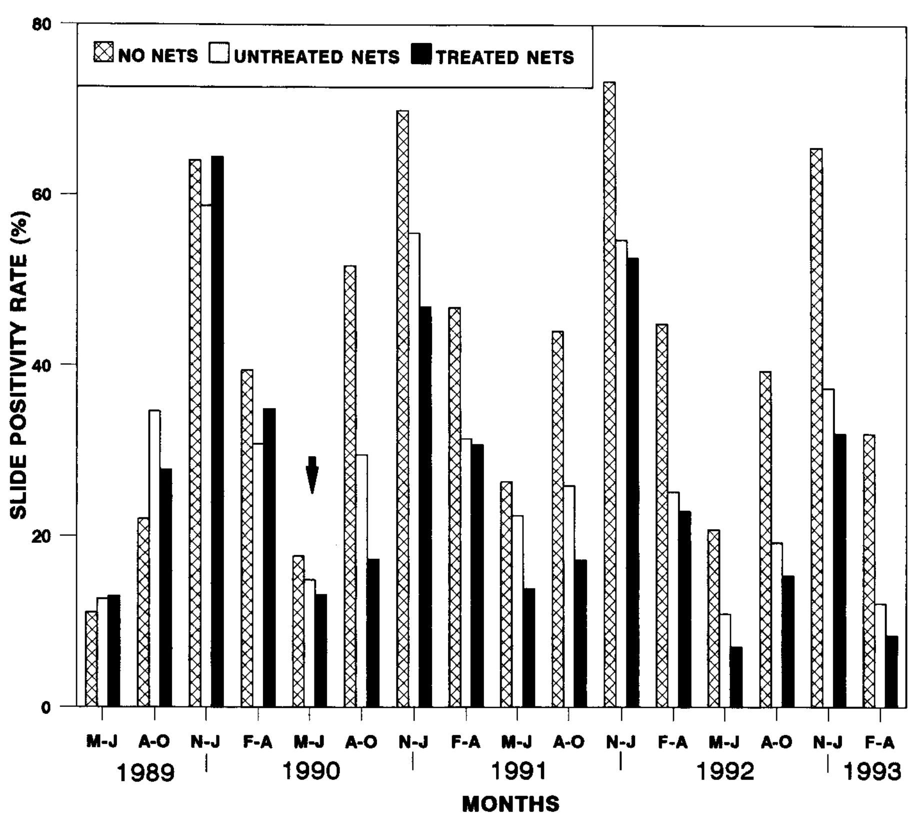

Fig. 1. Quarterly slide positivity rates in villages with lambdacyhalothrin-treated nets, untreated nets, and without nets. Arrow indicates beginning of the intervention in May 1990.

Table 2. Relative risk of malaria in villages with lambdacyhalothrin-treated nets (TN), untreated nets (UN), and no nets (NN).

Proportions	Relative risk (95% confidence interval) and P^1 values		
	API[2]	SPR[3]	SfR[4]
Baseline year			
TN/NN	1.21 (1.07; 1.44); $P < 0.05$	0.89 (0.77; 1.03); $P > 0.1$	0.95 (0.81; 1.12); $P > 0.5$
TN/UN	1.07 (0.93; 1.23); $P > 0.3$	1.07 (0.94; 1.21); $P > 0.2$	1.10 (0.96; 1.26); $P > 0.1$
UN/NN	1.13 (0.94; 1.35); $P > 0.1$	0.83 (0.72; 0.97); $P < 0.05$	0.86 (0.73; 1.02); $P > 0.05$
Intervention year 1			
TN/NN	0.38 (0.33; 0.44); $P < 0.001$	0.56 (0.48; 0.64); $P < 0.001$	0.55 (0.47; 0.65); $P < 0.001$
TN/UN	0.61 (0.52; 0.72); $P < 0.001$	0.78 (0.67; 0.90); $P < 0.001$	0.74 (0.63; 0.88); $P < 0.001$
UN/NN	0.62 (0.55; 0.70); $P < 0.001$	0.71 (0.64; 0.80); $P < 0.001$	0.74 (0.65; 0.84); $P < 0.001$
Intervention year 2			
TN/NN	0.46 (0.39; 0.54); $P < 0.001$	0.57 (0.49; 0.66); $P < 0.001$	0.58 (0.49; 0.69); $P < 0.001$
TN/UN	0.83 (0.69; 0.99); $P < 0.05$	0.86 (0.73; 1.01); $P > 0.05$	0.87 (0.73; 1.04); $P > 0.1$
UN/NN	0.55 (0.47; 0.64); $P < 0.001$	0.66 (0.58; 0.75); $P < 0.001$	0.67 (0.57; 0.77); $P < 0.001$
Intervention year 3			
TN/NN	0.33 (0.26; 0.42); $P < 0.001$	0.39 (0.32; 0.49); $P < 0.001$	0.39 (0.31; 0.50); $P < 0.001$
TN/UN	0.71 (0.55; 0.91); $P < 0.01$	0.79 (0.63; 1.00); $P > 0.05$	0.84 (0.65; 1.10); $P > 0.2$
UN/NN	0.46 (0.38; 0.56); $P < 0.001$	0.50 (0.42; 0.59); $P < 0.001$	0.46 (0.38; 0.57); $P < 0.001$

[1] χ^2 test.
[2] API, annual parasite index. Malaria cases per 1,000 population.
[3] SPR, slide positivity rate (%). Proportion of malaria cases among all febrile cases.
[4] SfR, slide falciparum rate (%). Proportion of *Plasmodium falciparum* cases among all febrile cases.

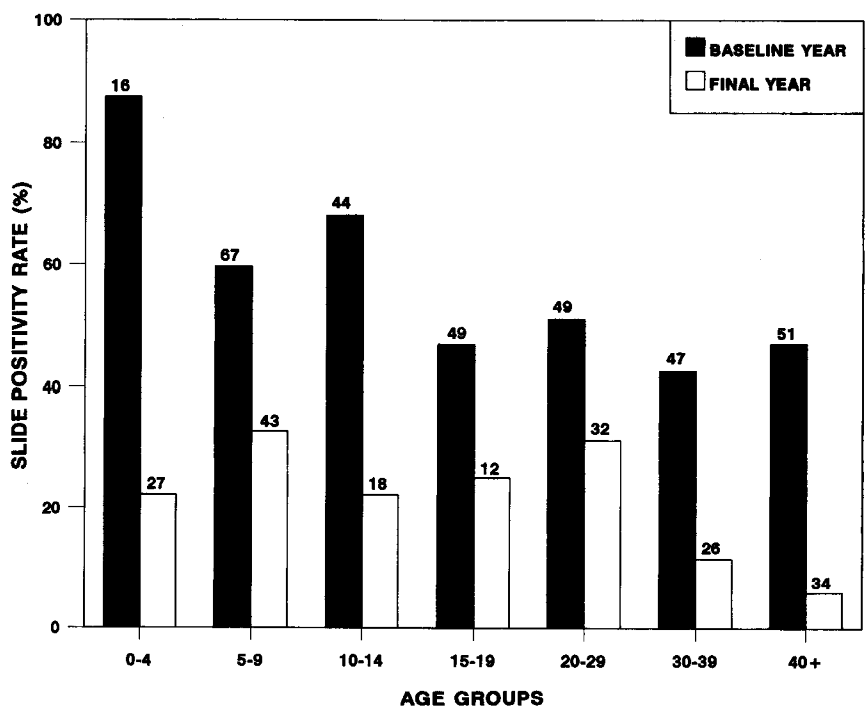

Fig. 2. Changes in the slide positivity rate by age from baseline year to last year of the trial in the villages with lambdacyhalothrin-treated nets. Numbers of slides are shown on top of each bar.

Table 3. Malaria parasite rates and relative risk based on cross-sectional surveys conducted in January 1990 (baseline), 1991, and 1992 (χ^2 test).

Intervention	1990	1991	1992
Parasite rate (%)			
No nets (NN)	23/77 (29.9%)	46/103 (44.7%)	44/258 (17.1%)
Untreated nets (UN)	35/188 (18.6%)	41/230 (17.8%)	25/223 (11.2%)
Treated nets (TN)	58/283 (20.5%)	23/174 (13.2%)	12/151 (7.9%)
Relative risk (95% CI)			
TN/NN	0.69 (0.45; 1.03) $P > 0.05$	0.29 (0.19; 0.46) $P < 0.0001$	0.46 (0.25; 0.85) $P < 0.01$
TN/UN	1.10 (0.75; 1.60) $P > 0.6$	0.74 (0.46; 1.19) $P > 0.2$	0.71 (0.37; 1.37) $P > 0.2$
UN/NN	0.62 (0.39; 0.98) $P < 0.05$	0.40 (0.28; 0.57) $P < 0.001$	0.66 (0.42; 1.04) $P > 0.05$

groups, in which the reductions were nonsignificant. Reduction in SPR was largest for the under-5-year-old age group.

In villages without nets observed malaria parasite rates in mass blood surveys without regard to fever increased during 1991 but declined in 1992 compared with the 1990 (baseline) survey (Table 3). In the other 2 areas parasite rates declined in both the subsequent years. Based on the 1992 survey, the relative risk of malaria with treated nets vs. no nets was 0.46 and the risk difference was significant ($P < 0.01$). Relative risk with treated nets vs. untreated nets was 0.71 and with untreated nets vs. no nets was 0.66, but the difference was not significant ($P > 0.05$).

An apparent increase occurred in the rate of spenomegaly and AES in children in the villages without nets or untreated nets compared with a reduction in both the parameters in the villages with treated nets (Table 4). Relative risk of splenomegaly as seen from the 1993 survey between treated net vs. no net, treated net vs. untreated net, and untreated net vs. no net groups was 0.065, 0.101, and 0.643, respectively, and the differences were statistically significant.

The proportions of children with various grades of anemia (such as severe, moderate, and mild) and the mean Hb concentrations as determined by surveys in September 1991 (i.e., the first year of intervention) and February 1993 in the villages without nets, with untreated nets, and with treated nets, are given in Table 5. From the results of the February 1993 survey, no significant difference occurred in the anemia rate ($P > 0.9$) or mean Hb concentration ($P > 0.1$) between the villages with untreated nets and those without nets. However, both these parameters were significantly lower ($P < 0.0001$) in the villages with treated nets compared with those in either untreated net or no net villages.

DISCUSSION

In rural India the most malarious areas are the foothills, forested hills, and forest fringe areas. In forested hills in Orissa State more than 77% of malaria infections are due to *P. falciparum* alone (Yadav et al. 1990). The only method applied for vector control in forest areas, especially in Orissa, is indoor residual spraying with DDT. Poor coverage because of refusals by householders and frequent mud plastering of sprayed walls have caused serious setbacks to the spraying program. Our experience with the application of bioenvironmental measures in forested villages was not encouraging because of inaccessibility of vector breeding habitats. The health infrastructure in the forest areas is generally inadequate. Therefore, among the new al-

Table 4. Impact of bednet usage on the rate of splenomegaly in children aged 2–9 years based on cross-sectional surveys conducted in February each year.

	Parameter[1]	1990	1991	1993[2]
No nets (NN)	Splenomegaly	21/67 (31.3%)	Not done	36/73 (49.3%)
	AES	1.4		1.7
Untreated nets (UN)	Splenomegaly	Not done	14/73 (19.2%)	20/63 (31.7%)
	AES		1.8	2.0
Treated nets (TN)	Splenomegaly	9/54 (16.7%)	Not done	2/62 (3.2%)
	AES	1.8		1.5

[1] AES, average grade of enlarged spleen.
[2] Relative risk (R) of splenomegaly in 1993 was: TN/NN: R = 0.065 (0.016; 0.259); $\chi^2 = 35.2$, $P < 0.001$; TN/UN: R = 0.101 (0.024; 0.413); $\chi^2 = 17.5$, $P < 0.001$; UN/NN: R = 0.643 (0.418; 0.989); $\chi^2 = 4.31$, $P < 0.05$.

Table 5. Impact of the interventions on the prevalence of anemia in children based on cross-sectional surveys.[1]

Intervention	n	% anemia: Severe (<7 g Hb/dl)	Moderate (7–10 g Hb/dl)	Mild[2]	Total	Mean Hb concentration[3] (g/dl) ± SD
September 1991 survey						
No nets	67	56.7	40.3	3.0	100.0	7.2 ± 1.5
Untreated nets	87	26.4	57.5	3.4	87.3	8.2 ± 2.4
Treated nets	70	4.3	47.1	17.1	68.5	10.3 ± 2.5
February 1993 survey[4]						
No nets (NN)	72	4.2	29.2	15.3	48.7	11.0 ± 2.4
Untreated nets (UN)	70	7.1	25.7	14.3	47.1	11.6 ± 3.3
Treated nets (TN)	70	0.0	2.9	4.3	7.2	14.5 ± 2.1
DDT	88	29.5	63.3	5.7	98.5	7.6 ± 1.3

[1] Hb, hemoglobin.
[2] Mild anemia: <11 g Hb/dl for children 6 months to 5 years of age and <12 g Hb/dl for children 6–14 years of age.
[3] Hb concentration in 1993 survey: NN vs. UN: $t = 1.17$, $P > 0.1$, df = 140; TN vs. UN: $t = 6.28$, $P < 0.001$, df = 138; and TN vs. NN: $t = 9.33$, $P < 0.001$, df = 140.
[4] Significance of differences in anemia levels (df = 2): NN vs. UN: $\chi^2_{M\text{-}H} < 0.001$, $P > 0.9$; TN vs. UN: $\chi^2_{M\text{-}H} = 21.4$, $P < 0.001$; TN vs. NN: $\chi^2_{M\text{-}H} = 23.06$, $P < 0.001$; and TN vs. DDT: $\chi^2_{M\text{-}H} = 75.8$, $P < 0.001$.

ternatives, the efficacy of personal protection measures, such as impregnated bednets, against malaria needed evaluation.

From the results of these trials it is clearly evident that the treated bednets caused significant reductions in the incidence of malaria and other clinical measures (rates of splenomegaly and anemia in the children). These results are similar to those reported previously (Bradley et al. 1986, Snow et al. 1988, Lyimo et al. 1991, Jana-Kara et al. 1995). In southern Orissa a contemporary 2-year study also reported a reduction in malaria following introduction of lambdacyhalothrin-treated nets (Das et al. 1993). However, the present study covered a longer period of 4 years, including the baseline year. In Sichuan Province, China, large-scale spraying of bednets with deltamethrin resulted in marked reductions in malaria (Cheng et al. 1995). In a malaria endemic area of the Thai–Burmese border, permethrin-treated bednets were associated with a significant reduction in maternal malaria-associated anemia (Dolan et al. 1993).

Although a decrease occurred in SPR in all age groups, children under 5 years old constituted the most vulnerable group and in this particular group the decrease was most remarkable. In Papua New Guinea, Graves et al. (1987) reported marked declines in the incidence and prevalence of *P. falciparum* in under-5-year-old children due to use of permethrin-treated nets, but not in older children.

Untreated nets were better than no nets, but protection from malaria by treated nets was definitely superior, as found by Jana-Kara et al. (1995) and D'Alessandro et al. (1995). Weekly active morbidity surveillance and treatment for malaria were common in all 3 areas. Therefore, presumptive treatment for malaria was given to all febrile cases pending slide examination. This, together with improvement in case detection and treatment, might have presumably led to the observed drop in the parasite rate during the 2nd year in the villages without nets. Declines in malaria in the rate in the villages with treated and untreated nets were markedly higher than those in villages without nets. In conclusion, the bednets impregnated with lambdacyhalothrin were highly effective and offer a new alternative approach for fighting malaria, especially in the forested areas.

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