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EVALUATION OF LAMBDACYHALOTHRIN-IMPREGNATED BEDNETS IN A MALARIA ENDEMIC AREA OF INDIA. PART 2. IMPACT ON MALARIA VECTORS

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ABSTRACT. In malaria endemic villages of the Indian State of Orissa, the impact of bednets treated with lambdacyhalothrin at 25 mg/m^2 on malaria vectors was assessed during a 3-year intervention trial beginning in May 1990. The main malaria vector was *Anopheles culicifacies* with a small contribution from *Anopheles fluviatilis*. The impregnated bednets caused a significant reduction in vector density as assessed by morning indoor resting catches, man-biting rate, light trapping, the proportion of females engorged with human blood, and the parity rate as compared with villages with untreated or no nets. No statistically significant difference was observed in these parameters between the villages with untreated nets or no nets. The trial demonstrated that the lambdacyhalothrin-treated nets were highly effective against the malaria vectors.

KEY WORDS Bednets, lambdacyhalothrin, India, mosquitos, density, biting, parity

INTRODUCTION

Since the 1980s trials on the use of pyrethroid-impregnated bednets in several countries have reported significant impacts on malaria vectors. Bed nets impregnated with permethrin reduced the density, human blood index, and biting rate of *Anopheles farauti* Laveran in coastal Papua New Guinea (Charlwood and Graves 1987), reduced indoor density and man-biting of *Anopheles gambiae* Giles s.s. in The Gambia (Lindsay et al. 1989), and reduced survival and sporozoite rates in Tanzania (Magesa et al. 1991). In Suriname, exit traps fitted on a hut with permethrin-treated nets yielded fewer female *Anopheles darlingi* Root compared to huts with untreated nets (Rozendaal et al. 1989).

Use of cyfluthrin-impregnated bednets in mining settlements in the forested hills of Orissa, India, caused a significant reduction in the density and biting of the malaria vector *Anopheles fluviatilis* James (Yadav and Sharma 1994). Use of nets impregnated with deltamethrin reduced indoor density of *Anopheles sinensis* Wiedemann and *Anopheles anthropophagus* Xu and Feng in China (Li et al. 1989) and suppressed the biting and indoor density of *Anopheles minimus* Theobald in northeastern India (Jana-Kara et al. 1995). A study in Burkina Faso showed that impregnated bednets, even if considerably torn, limited human–anopheline contact (Carnevale et al. 1992).

In Orissa State of eastern India, the principal malaria vectors are *Anopheles culicifacies* Giles and *An. fluviatilis*. *Anopheles culicifacies* is a predominantly endophilic species in Orissa (Chand et al. 1993) and comprises mainly sibling species C (>70%) and B (28%), whereas species A is rare and species D has not been recorded from this area (N. Nanda et al., unpublished paper). *Anopheles fluviatilis* comprises mainly sibling species S (98%), T, and U (Subbarao et al. 1994). Species S is highly anthropophilic (human blood index 0.91) in this area (Nanda et al. 1996). Following our description of the trial area and implementation (Sampath et al. 1998), the present paper reports the impact of lambdacyhalothrin-impregnated bednets on the vector population compared to that in villages with untreated nets and no nets (control).

MATERIALS AND METHODS

Details on the demography and topography of the study area, study design, distribution and impregnation of the bednets, and acceptability of nets have been described by Sampath et al. (1998). In short, 3 areas were compared: villages with lambdacyhalothrin-treated nylon nets (treated at 25 mg/m^2), villages with untreated nets, and villages with no nets (control).

Morning resting catches

From each of the 3 study areas a representative index village was selected for entomological monitoring. Mosquito catching stations (2 single-room huts and 2 cattle sheds) consisted of 4 fixed catching stations plus 4 similar stations chosen randomly each time. Beginning in February 1990, catches of morning resting populations were made every 2 wk with aspirators and torches. A collector spent 15 min in each catching station searching for mosquitoes. The mean monthly density of indoor resting mosquitoes was calculated per man-hour of time spent. Bednets were provided in the respective villages in May 1990 and monitoring was done until April 1993.

For comparison of the density of *An. culicifacies* in single bedroom houses with treated or untreated nets, freshly treated nets were given to the occu-

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pants of 2 houses in January 1992, and the existing nets in 2 other similar houses (within the treated net area) were replaced with untreated nets. Catches were also made from 2 houses with one bedroom each in the village with untreated nets. Indoor resting catches were made every 2 wk from January 1992 until March 1993 from these 3 pairs of special study houses.

Trap collections

Mosquito populations were sampled monthly by Centers for Disease Control ultraviolet (UV) light traps (with a 4-W tube). Two light traps were hung in each index village, one inside a designated house and another at a fixed site outdoors, and operated from 1800 to 0600 h one night per month. Traps were hung about 1.5 m above ground level and about 50 cm from an occupied bednet or bed (in the control without a net).

Night-biting collections

Man-biting collections were done all night (1800–0600 h) once a month from November 1990 to October 1991. In each index village, 2 fixed houses were used for mosquito biting collections indoors and 2 fixed outdoor sites were used for mosquito biting collections outdoors. In the villages with treated or untreated nets, the volunteer human baits (one each in 2 houses and 2 outdoors) slept under the respective type of nets, the hems of which were lifted 30 cm above the beds, allowing access to mosquitoes (Jana-Kara et al. 1995). In the index village without nets, the human baits slept without nets. A team of 4 collected the mosquitoes landing on each of the 4 baits from 1800 to 0100 h and another team worked from 0100 to 0600 h.

Cattle-biting collections were also made concurrently in the control area. A calf was tied outdoors under a partially lifted untreated mosquito net. Bloodfed female mosquitoes were collected hourly as they rested inside the net after biting.

Classification of mosquitoes

Species of all the mosquitoes collected by the different methods were identified and recorded according to their abdominal state. Blood meals of *An. culicifacies* and *Culex quinquefasciatus* Say were identified by countercurrent electrophoresis using agar gel (1.5%) and human and bovine antisera (Joshi et al. 1988). *Anopheles culicifacies* females collected resting indoors, during the morning from February 1991 to January 1992, were dissected to determine parity.

RESULTS

Twenty-seven species of mosquitoes were recorded in the village with no nets. *Anopheles culicifacies* was the most common species, both in human dwellings (28.7% of total catch) and in cattle sheds (36.1%); *An. fluviatilis* constituted <2% of the catch (Table 1). Analysis of the all-night biting pattern on a man sleeping without nets showed that 86% of the total night's bites of *An. culicifacies* and 100% of those of *An. fluviatilis* took place from 2100 to 0400 h (Fig. 1). During this period 71% of the total night's bites of *An. culicifacies* took place on cattle. The remainder of bites took place during the evening and predawn periods.

Table 1. Percent composition of indoor resting mosquitoes based on morning catches from human dwellings (HD) and cattle sheds (CS) in the village without nets.

Species[1]	HD	CS
An. annularis Van der Wulp	3.28	17.62
An. culicifacies Giles	28.66	36.12
An. fluviatilis James	1.45	0.30
An. pallidus Theobald	4.92	14.98
An. subpictus Grassi	24.04	15.90
An. vagus Donitz	16.61	7.46
Other anophelines[2]	0.70	1.88
Cx. quinquefasciatus Say	17.48	2.44
Other culicines[3]	2.85	3.30
Total[4]	100%	100%

[1] *An.*, *Anopheles*; *Cx.*, *Culex*.

[2] *Anopheles aconitus* Donitz, *An. barbirostris* Van der Wulp, *An. jamesi* Theobald, *An. jeyporiensis* James, *An. nigerrimus* Giles, *An. ramsayi* Covell, *An. splendidus* Koidzumi, *An. tessellatus* Theobald, *An. theobaldi* Giles, and *An. varuna* Iyengar.

[3] *Culex bitaeniorhynchus* Giles, *Cx. brevipalpis* (Giles), *Cx. gelidus* Theobald, *Cx. fuscanus* Wiedemann, *Cx. mimulus* Edwards, *Cx. nigropunctatus* Edwards, *Cx. sinensis* Theobald, *Cx. tritaeniorhynchus* Giles, *Cx. vishnui* Theobald and *Armigeres obturbans* Walk.

[4] Total mosquitoes collected from HD = 3,112 and from CS = 12,053.

A Kruskal–Wallis test on biweekly indoor densities of *An. culicifacies* during the baseline period (February–April 1990) showed no significant difference between the 3 areas ($H = 1.81$, $P > 0.4$, df = 2). During the first intervention year, beginning in May 1990, the density in rooms with treated nets in the villages with treated nets remained similar to or marginally lower than those in the other villages (Fig. 2). During the 2nd and 3rd years indoor resting densities in the treated net area were markedly lower than in the untreated net and no net areas, where they remained comparable with each other.

Kruskal–Wallis tests confirmed that between February and April of the 3rd year of intervention the densities in treated net villages were significantly lower than in those with untreated nets ($H = 7.65$, $P < 0.01$, df = 1) or no nets ($H = 8.48$, $P < 0.01$, df = 1). However, no significant difference in the densities occurred between villages with untreated nets and those with no nets ($H = 2.45$, $P > 0.1$, df = 1).

Within the treated net index village, morning

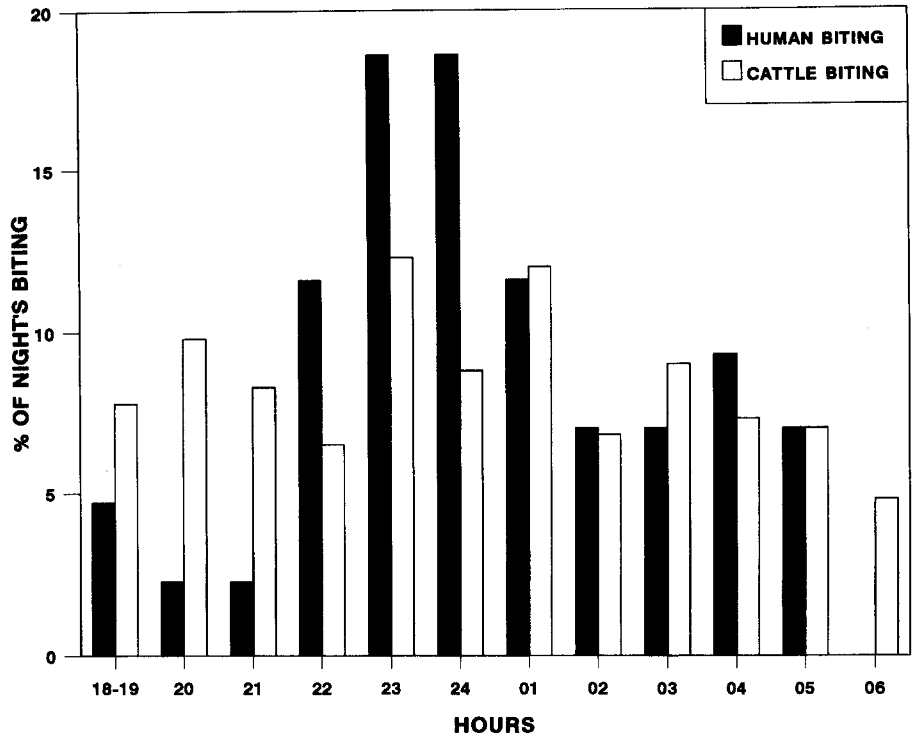

Fig. 1. Biting cycle of *Anopheles culicifacies* on humans and cattle. Percentages of the entire nights' catches are given on the *y* scale.

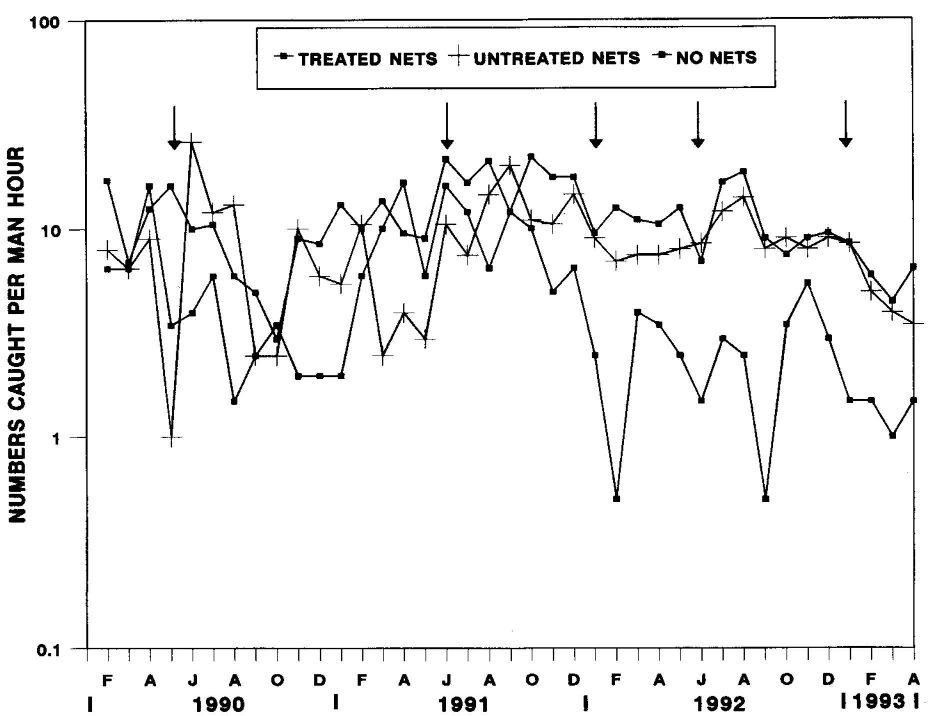

Fig. 2. Indoor resting density of *Anopheles culicifacies* in villages with lambdacyhalothrin-treated nets, untreated nets, and no nets. Note that *y* axis is a log scale. Arrows indicate bednet impregnation dates.

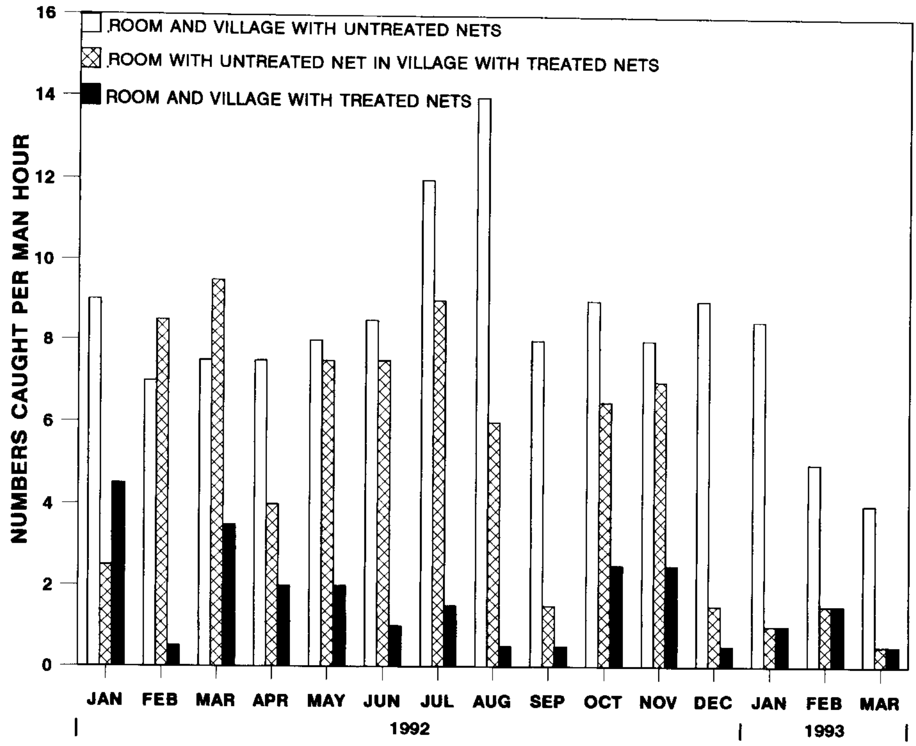

Fig. 3. Indoor resting density of *Anopheles culicifacies* based on morning catches from rooms with lambdacyhalothrin-treated nets or untreated nets in the villages with treated nets, and from the rooms with untreated nets in the village with untreated nets.

resting densities of *An. culicifacies* were significantly lower in the rooms with treated nets ($H = 7.69$, $P < 0.01$, df = 1) than in rooms where people occupied untreated bednets (Fig. 3). The latter were also significantly lower than in those in the untreated net village ($H = 7.10$, $P < 0.01$, df = 1), reflecting a mass control effect in the village with treated nets.

Anopheles culicifacies collected by UV light traps in villages with treated nets, untreated nets, or no nets totaled 25, 62, and 125, respectively. A Kruskal–Wallis test on log-transformed data showed no significant difference between light trap density in villages with untreated nets versus no nets ($H = 3.27$, $P > 0.05$, df = 1). However, significantly fewer *An. culicifacies* were trapped in the village with treated nets compared to those with untreated nets ($H = 6.31$, $P < 0.02$, df = 1) or no nets ($H = 10.98$, $P < 0.001$, df = 1). No *An. fluviatilis* were present in trap collections in the treated net village, although traps in untreated net and no net villages yielded 2 and 40 *An. fluviatilis,* respectively.

The numbers of *An. culicifacies* caught in 12 nights of man-biting collections in villages with treated nets, untreated nets, and no nets were 2, 18, and 43, respectively. Analysis of the log-transformed all night biting collections of *An. culicifacies* by Kruskal–Wallis tests showed no statistically significant difference in vector bites on humans under partially lifted untreated nets or no nets ($H = 2.53$, $P > 0.1$, df = 1). However, significantly fewer mosquitoes bit under treated nets as compared with untreated nets ($H = 8.63$, $P < 0.005$, df = 1) or no nets ($H = 12.01$, $P < 0.001$, df = 1). The number of *An. fluviatilis* caught biting human baits in those areas was 0 (treated), 1 (untreated), and 20 (no nets), respectively.

Data on the anthropophilic index (AI = percentage of human blood feeds) are given in Table 2. No significant difference was found between the AI of *An. culicifacies* ($P > 0.2$) or *Cx. quinquefasciatus* ($P > 0.1$) in the untreated net and no net villages. However, the AI of *An. culicifacies* in treated net villages was significantly lower than that in the untreated net ($P < 0.002$) or no net ($P < 0.0001$) villages. Similarly, the AI of *Cx. quinquefasciatus* in the treated net villages was also significantly lower than in the untreated net or no net villages ($P < 0.0001$).

Monthly parity rates of *An. culicifacies* are shown in Fig. 4. The proportions of parous females in villages with treated nets, untreated nets, or no nets were 0.34, 0.44, and 0.43, respectively. The parity rate in the treated net village was significantly lower than in villages with either untreated nets ($\chi^2 = 11.1$, $P < 0.001$, df = 1) or no nets ($\chi^2 = 11.9$, $P < 0.001$, df = 1). No significant differ-

Table 2. Impact of intervention on anthropophilic index (AI) of *Anopheles culicifacies* and *Culex quinquefasciatus*.[1]

	An. culicifacies				*Cx. quinquefasciatus*			
Intervention	*n*	H+	C+	AI (%)	*n*	H+	C+	AI (%)
No nets (NN)	1,190	31	1,041	2.6	145	58	98	40.0
Untreated nets (UN)	1,030	19	911	1.8	159	49	120	30.8
Treated nets (TN)	880	2	811	0.2	186	19	140	10.2
Significance of difference (χ^2; df = 1)								
UN vs. NN	$\chi^2 = 1.12$ ($P > 0.2$)				$\chi^2 = 2.41$ ($P > 0.1$)			
TN vs. UN	$\chi^2 = 9.97$ ($P < 0.002$)				$\chi^2 = 21.71$ ($P < 0.001$)			
TN vs. NN	$\chi^2 = 16.75$ ($P < 0.001$)				$\chi^2 = 38.84$ ($P < 0.001$)			

[1] H+, human blood; C+, cattle blood. Mixed blood meals are added to each group; mosquitoes scoring negative for both are omitted.

ence was observed between the parity rates in untreated net and no net villages ($\chi^2 < 0.1$, $P > 0.9$, df = 1).

Among the indoor resting female *An. culicifacies*, the proportion of unfed females in the treated net village was significantly higher than those in the untreated net ($\chi^2 = 11.71$, $P < 0.001$, df = 1) or no net ($\chi^2 = 12.89$, $P < 0.001$, df = 1) villages (Table 3).

In UV light trap collections from the villages with treated nets, most female *An. culicifacies* were unfed (60%) and their proportion was significantly higher than in the collections from untreated net ($\chi^2 = 14.67$, $P < 0.001$, df = 1) or no net ($\chi^2 = 27.07$, $P < 0.001$, df = 1) villages. No significant difference existed in the proportions of unfed females either in the indoor resting ($\chi^2 = 0.02$, $P > 0.8$, df = 1) or trap collections ($\chi^2 = 0.30$, $P > 0.5$, df = 1) between the villages with untreated nets and no nets.

DISCUSSION

In Orissa State malaria is transmitted mainly by *An. culicifacies* and *An. fluviatilis*. The former species is found both in villages in forested and partially cleared areas, whereas the latter is more confined to the forests. *Anopheles culicifacies* was the main vector species in the study villages, with a small contribution from *An. fluviatilis* during cool,

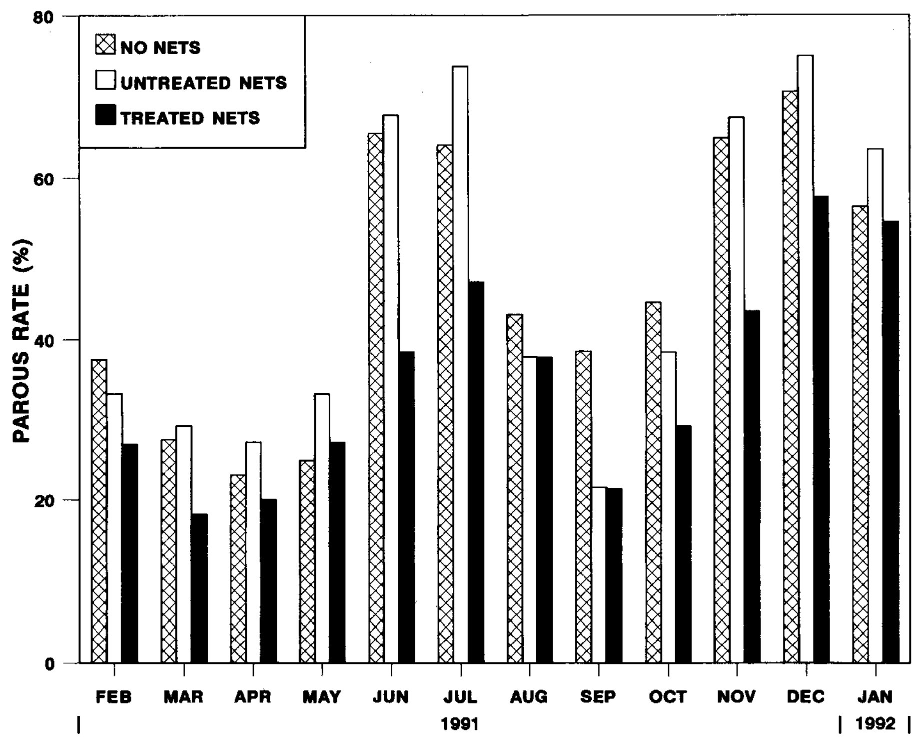

Fig. 4. Parity rates of *Anopheles culicifacies* in villages with lambdacyhalothrin-treated nets, untreated nets, and no nets.

Table 3. Abdominal appearance of *Anopheles culicifacies* caught resting indoors and by light traps.

Type of collection	*n*	Abdominal appearance (%) Unfed	Fed	Semi-gravid	Gravid
Resting collections					
No nets	952	1.2	46.7	28.9	23.1
Untreated nets	854	1.4	52.5	27.1	18.9
Treated nets	415	4.7	46.6	28.9	19.8
Trap collections					
No nets	125	12.0	47.2	19.2	21.6
Untreated nets	62	16.1	56.5	17.7	9.7
Treated nets	25	60.0	24.0	12.0	4.0

dry months. *Anopheles culicifacies* is known to be a predominantly endophilic species in the area, resting more in cattle sheds than in human dwellings (Chand et al. 1993). In our study all-night biting collections showed that most of the biting of *An. culicifacies* took place between 0900 and 0500 h. Because this was also the main sleeping time of the local people, the biting rhythm of *An. culicifacies* in this area is suitable for use of impregnated bednets. Our results are in agreement with those of Reuben et al. (1984) who reported no marked seasonal shift in feeding time of *An. culicifacies* in southern India, where this species took 72% of the total bites between 0900 and 0500 h. About 80% of total night bites of *An. culicifacies* were reported during a similar period in Bastar, Madhya Pradesh (Kulkarni 1987). In western India *An. culicifacies* exhibited a marginal seasonal variation in peak biting activity but in most months, except January and February winter months, the peaks remained confined between 0900 and 0500 h when two thirds of the total bites took place (Bhatt et al. 1991).

Lambdacyhalothrin-impregnated bednets reduced the densities of *An. culicifacies* and *An. fluviatilis* in the villages with treated bednets compared with those with untreated or no nets. In the previous paper (Sampath et al. 1998) we reported that compliance with bednet usage was satisfactory. Therefore, the general reduction in vector density in the villages with treated nets could be attributed partly to a mass killing effect resulting from community usage of impregnated bednets. The reduction of *An. culicifacies* resting indoors in rooms with impregnated nets might also reflect a possible excito-repellent effect of the pyrethroids. Reductions of the indoor densities of *An. farauti* (Charlwood and Graves 1987) and *An. gambiae* (Lindsay et al. 1989) by introduction of permethrin-impregnated bednets, of *An. gambiae* by permethrin- or lambdacyhalothrin-treated nets (Magesa et al. 1991), of *An. fluviatilis* by cyfluthrin-treated bednets (Yadav and Sharma 1994, Sharma and Yadav 1995), and of *An. minimus* by deltamethrin-treated bednets (Jana-Kara et al. 1995) have been reported earlier.

A slow increase to the full effect of bed-nets on the room density of *An. culicifacies* occurred during the first year of intervention, as shown in Fig. 2. This was probably partly because of the time taken by people in becoming habituated to the use of their nets and also partly because the 2nd impregnation of nets was done 1 year after the first impregnation, whereas impregnation every 6 months was required, as was learned later through bioassays during the first intervention year. When reimpregnation was done every 6 months and the bednet use rate improved, the full effect on vector density became manifest during the later 2 years of the trial.

The proportion of unfed female *An. culicifacies* indoors was relatively high in the villages with treated nets. A large proportion of unfed females also occurred in light traps near the baits under partially lifted treated bednets compared with untreated nets or no nets. This reduction in feeding success is attributed to prevention of feeding due to excito-repellency or deterrence caused by widespread use of impregnated bednets.

Because the biting time of *An. culicifacies* coincided well with the sleeping time of the local people, the use of bednets greatly reduced human–mosquito contact and markedly suppressed the man-biting rate. Whether a marginal behavioral shift to early evening biting by unfed females that failed to feed on the previous night occurred could not be ascertained. Such a shift was found by Charlwood and Graves (1987) in *An. farauti*, but seems less likely under our circumstances in Orissa because *An. culicifacies* is a predominantly zoophilic species and cattle were readily available as alternative hosts. Diversion to cattle was evident from reduction in the AI (proportion of females engorged with human blood) of the surviving population of *An. culicifacies* and of the filariasis vector *Cx. quinquefasciatus*. Widespread usage of lambdacyhalothrin-treated nets seems to have diverted a sizeable proportion of hungry female mosquitoes to cattle, resulting in reduction in the AI. Charlwood and Graves (1987) reported a similar reduction in the human blood index of *An. farauti* by use of permethrin-treated bednets in Papua New Guinea and diversion of the biting to dogs.

In the control village (no nets) about one third of the indoor resting population of *An. culicifacies* had ovaries in Christophers' stage V, which is consistent with the endophilic habit of the species. Large proportions of semigravid and gravid females in morning collections indoors further support this observation. Predominantly indoor resting species of mosquitoes are expected to be more likely to be killed by impregnated nets. As expected, the parity rates of *An. culicifacies* were significantly lower in the villages with treated nets than in those with untreated or no nets.

Under the circumstances of our study in Orissa, the use of untreated nets compared with no nets had little entomological advantage, except that the nets provide physical protection from mosquito bites if tucked in properly and used carefully as shown by a small but statistically nonsignificant reduction in the AI in villages with untreated nets compared to those with no nets. Thus, although untreated nets did not cause a drop in vector density or parity rate, some degree of protection from malaria was expected by their widespread and proper usage. This trial demonstrated that lambdacyhalothrin-treated bednets had a definite advantage over untreated nets or no nets and that these nets were highly effective against the vectors of malaria in Orissa.

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