EVALUATION OF LAMBDACYHALOTHIN-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/m² 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, mosquitoes, 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. Bednets 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 Burkins 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/m²), 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-
pant's 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 corded in the village with no nets. Anopheles culicifacies females collected resting indoors, during the morning from February 1991 to January 1992, were dissected to determine parity.

**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.

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 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

**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.**

<table>
<thead>
<tr>
<th>Species</th>
<th>HD</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>An. annularis</em> Van der Wulp</td>
<td>3.28</td>
<td>17.62</td>
</tr>
<tr>
<td><em>An. culicifacies</em> Giles</td>
<td>28.66</td>
<td>36.12</td>
</tr>
<tr>
<td><em>An. fluviatilis</em> James</td>
<td>1.45</td>
<td>0.30</td>
</tr>
<tr>
<td><em>An. pallidus</em> Theobald</td>
<td>4.92</td>
<td>14.98</td>
</tr>
<tr>
<td><em>An. subpictus</em> Grassi</td>
<td>24.04</td>
<td>15.90</td>
</tr>
<tr>
<td><em>An. vagus</em> Donitz</td>
<td>16.61</td>
<td>7.46</td>
</tr>
<tr>
<td>Other anophelines</td>
<td>0.70</td>
<td>1.88</td>
</tr>
<tr>
<td><em>Cx. quinquefasciatus</em> Say</td>
<td>17.48</td>
<td>2.44</td>
</tr>
<tr>
<td>Other culicines</td>
<td>2.85</td>
<td>3.30</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

1 _An._, _Anopheles_; _Cx._, _Culex_.
2 _Anopheles aconitus_ Donitz, _An. barbirostris_ Van der Wulp, _An. jamesi_ Theobald, _An. jeyporensis_ James, _An. nigerrimus_ Giles, _An. ramusci_ Covell, _An. splendidas_ Koidzumi, _An. tessellatus_ Theobald, _An. theobaldi_ Giles, and _An. varuna_ lyengar.
3 _Culex bitaeniorhynchus_ Giles, _Cx. brevipalpis_ (Giles), _Cx. gelidus_ Theobald, _Cx. fuscans_ Wiedemann, _Cx. minus_ Edwards, _Cx. nigropunctatus_ Edwards, _Cx. sinensis_ Theobald, _Cx. tritaeniorynchus_ Giles, _Cx. vishnui_ Theobald and _Armigeres obturans_ Walk.
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.
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

<table>
<thead>
<tr>
<th>Intervention</th>
<th>An. culicifacies</th>
<th>Cx. quinquefasciatus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>H+</td>
</tr>
<tr>
<td>No nets (NN)</td>
<td>1,190</td>
<td>31</td>
</tr>
<tr>
<td>Untreated nets (UN)</td>
<td>1,030</td>
<td>19</td>
</tr>
<tr>
<td>Treated nets (TN)</td>
<td>880</td>
<td>2</td>
</tr>
</tbody>
</table>

Significance of difference ($\chi^2$; df = 1)
- UN vs. NN: $\chi^2 = 1.12 (P > 0.2)$
- TN vs. UN: $\chi^2 = 9.97 (P < 0.002)$
- TN vs. NN: $\chi^2 = 16.75 (P < 0.001)$

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.
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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