Medical and Veterinary Entomology (2005) **19**, 286–292

Village-scale evaluation of mosquito nets treated with a tablet formulation of deltamethrin against malaria vectors

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Abstract. A field trial was carried out in the Sundargarh district of Orissa, India on the efficacy of mosquito nets treated with a tablet formulation of deltamethrin (K-O TAB®) against malaria vectors. Treated nets were used in one village, and in the two control villages, one used untreated nets and the other used indoor spraying with DDT, without nets. In this area the primary malaria vectors are *Anopheles culicifacies* Giles *sensu lato* (Diptera: Culicidae) and *An. fluviatilis* James *s.l.*, which are both endophagic and endophilic, and fully susceptible to deltamethrin. Treatment of a 10-m^2 mosquito net with one of the tablets gave a deltamethrin deposit of 25 mg/m^2. Bioassays repeated on domestically used nets over 7 months showed persistence of almost 100% mortality of *An. fluviatilis*, whereas *An. culicifacies* showed a decline from 100% to 71% mortality over this period, after which the nets were re-treated and bioassays were not continued. The sum of collections of mosquitoes resting in village houses and those in exit traps and dead on floor sheets showed a reduction in the numbers of the two vector species due to the treated nets, compared with untreated or no nets, but no reduction in other anophelines or *Culex* species. Large proportions of the collections of the vector and non-vector anophelines were dead on the floor sheets, but among *Culex*, mortality was delayed. Treated and untreated nets reduced the proportion of anophelines that had blood-fed; the treated nets did so more effectively than the untreated in the case of *An. culicifacies* and of *Culex* mosquitoes. In rooms with treated nets a larger proportion of the total collections [dead + live] were in the exit traps, which can be attributed to the excito-repellent effect of deltamethrin. It is easier to pack and handle tablets of insecticide than liquid concentrate and the use of one tablet per net may be preferable to making up a large volume of diluted insecticide and dipping many nets at a time.

Key words. *Anopheles culicifacies*, *Anopheles fluviatilis*, bioassays, deltamethrin (K-O TAB®), excito-repellency, insecticide-treated nets, malaria vector control, mosquito mortality, tablet prevention of blood feeding.

Introduction

Since its establishment in 1958, the Indian National Malaria Eradication Programme has relied mainly on indoor residual spraying with DDT. The first report of tolerance to DDT in the primary malaria vector *Anopheles culicifacies* Giles *sensu lato* was from Maharashtra State by Rahman *et al.* (1959). Continuous

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usage of this and other insecticides for house spraying has resulted in the development of resistances to DDT, HCH and malathion in *An. culicifacies*, which is responsible for the transmission of 60–70% of the malaria in India (Sharma, 1998). Synthetic pyrethroids such as deltamethrin, lambdacyhalothrin and cyfluthrin have been evaluated in the field for their effectiveness against disease vectors (Ansari *et al.*, 1986; Singh *et al.*, 1989; Yadav *et al.*, 1996). These were introduced during the 1990s in some parts of India to control multiple-resistant malaria vectors in high-risk malarious areas.

Mosquito nets treated with synthetic pyrethroids have proved to be an important tool for the control of malaria and other vector borne diseases (Lengeler, 2000). In India trials of insecticide-treated nets carried out in Assam and Orissa have shown promising results against malaria transmitted by *An. minimus* Theobald (Jana-Kara *et al.*, 1995), *An. culicifacies* (Sampath *et al.*, 1998; Yadav *et al.*, 2001), and *An. fluviatilis* James *s.l.* (Sharma & Yadav, 1995). Insecticide-treated mosquito nets are now one of the operational strategies recommended by the National Vector Borne Disease Control Programme of the Government of India.

At present mosquito nets are treated either with emulsifiable concentrates (EC), flowable or suspension concentrates (SC), microemulsions (ME) or microcapsule suspensions (CS) of synthetic pyrethroids. These formulations need to be measured volumetrically (avoiding skin contact with the concentrates) to make aqueous mixtures for dipping batches of nets in the field. It may be preferable to use a deltamethrin tablet formulation (K-O TAB®), which has been widely used outside India. One tablet is intended to provide the dose for one net. This study reports various entomological measurements in rooms in a village provided with K-O TAB®-treated nets compared with a village with untreated nets and another with no nets but with DDT spraying.

Materials and methods

Study area

The study was conducted in the villages of Birkera, Dudurta and San Pokhari, which are ~6 km from Bisra Community Health Centre in the Sundargarh district in the northern part of Orissa state, India. These villages are located within 25–35 km of Rourkela City and are accessible throughout the year. In all three villages the primary malaria vectors are *An. culicifacies* and *An. fluviatilis*. The former breeds in ponds, pools and rice fields, whereas the later breeds exclusively in slow-flowing streams. The annual rainfall is between 160 and 200 cm, falling mainly from mid-June to September, and temperatures range from 10 to 45°C. The vector populations are much higher from August to February than for the rest of the year.

The villagers are poor and live in thatched-roof houses clustered in small hamlets either in the forest area or on deforested land. Ethnic tribal communities constitute 98% of the village populations. Their economy is dependent on forest products and subsistence farming. Malaria is a major public health problem in the area.

Selection of study villages

On the basis of available epidemiological data from the Bisra Community Health Centre, some villages were shortlisted and preliminary rapid fever and entomological surveys were carried out. On this basis, three villages were selected and randomly assigned as the trial village (Birkera) to receive treated nets, a village to receive untreated nets (Dudurta) and a control village that received no nets (San Pokhari). Birkera consisted of 92 houses scattered in four hamlets, with a population of 506. Dudurta had 271 people and San Pokhari had 367. Indoor residual spraying with DDT was discontinued in 2003 during the trial in Birkera and Dudurta, but was carried out in March 2003 in San Pokhari.

Mosquito nets and their distribution

The mosquito nets were of polyester polyfilament fibre, 100-denier strength, white in colour, 156-mesh size (12×13 holes/in^2). The total surface area of the net was 10 m^2. Before the start of the trial, village meetings were organized to inform inhabitants about proper and regular use of nets and of the aims and importance of the study. Nets were distributed free of charge based on a survey of where people slept and was carried out in the first week of December 2002. The number of nets distributed to each household was recorded in a register and signatures of the recipients were obtained. In total 283 mosquito nets treated with the tablet formulation of deltamethrin and 137 untreated nets were distributed in Birkera and Dudurta, respectively. The study population was requested not to wash the nets during the study and this was complied with very well. The project staff, in consultation with panchayat members (governing council) and other opinion leaders, constituted a village committee to monitor proper use and maintenance of the mosquito nets.

Treatment of nets

The deltamethrin tablets (K-O TAB®), each weighing 1 g and containing 250 mg of active ingredient, were supplied free of charge by M/S Aventis Crop Science India Limited Mumbai, India. Mosquito nets were treated with K-O TAB® at a dosage of 25 mg/m^2. One tablet was mixed with 300 ml of water, which is sufficient to wet a 10-m^2 polyester net, and only one net was dipped in the mixture. Project staff demonstrated the impregnation process and subsequently nets were treated by householders under the supervision of the project team. Normal precautions, such

as use of rubber gloves, avoiding contact with eyes, nose and mouth, and washing of hands thoroughly after impregnation, were followed. The nets treated with insecticide were laid on a non-absorbent plastic sheet in the shade and allowed to become partially dry, after which the nets were hung on a wire in the shade to dry completely. Re-treatment of nets was carried out in June 2003, 6 months after the first treatment.

Periods of data collection

After selection of the study villages, pre-intervention mosquito collections were carried out from August to November 2002. Post-intervention data were collected by all of the methods described below from December 2002 to May 2003, with house-resting catches continuing until December 2003.

Susceptibility of malaria vectors

The insecticide susceptibility status of wild caught adult *An. culicifacies* and *An. fluviatilis* against DDT (4%), malathion (5%) and deltamethrin (0.05%) was determined by standard 1-h exposures to insecticide-impregnated papers, followed by 24-h holding (W.H.O., 1975, 1998).

Bioassays on nets

The persistence of the insecticide on nets in regular use was determined by contact bioassays using a standard procedure (WHO, 1998). The tests were performed immediately after net treatment and thereafter at monthly intervals. Wild caught fully fed female *An. culicifacies* or *An. fluviatilis* were used in the bioassays. The mosquitoes were introduced into plastic cones attached to the netting with rubber bands and exposed for 3 min. The number of mosquitoes that were knocked down by the end of the exposure period and the mortality rate after 24 h were recorded. Five replicates, each with 10 mosquitoes, were exposed on each side of the net.

Mosquito collections

Adult mosquitoes were collected between 06.00 and 09.00 h monthly in four regularly used sentinel houses and in four other houses selected randomly on each occasion. The collections were in rooms with treated nets in Birkera, in rooms with untreated nets in Dudurta and in rooms with no nets, but subjected to DDT spraying, in San Pokhari. Thus, the collections were presumably influenced by insecticidal and excito-repellent effects of the nets and the amount of insecticide present in the collection rooms, as well as any 'mass effects' of community-wide use of the insecticides. All collected mosquitoes were identified to species and abdominal condition and held for 24 h to record any delayed mortality. The collection methods were as follows.

Floor sheet collection. Dead and moribund mosquitoes were collected from floor sheets that had been laid in the rooms the previous evening.

Exit trap collection. Live and dead mosquitoes were collected from rectangular exit traps measuring $35 \times 35 \times 35$ cm, with a conical cone of plastic material with an orifice of 1 cm^2, which had been fitted over one window the previous evening in the four sentinel houses in Birkera and Dudurta.

Hand catches of resting mosquitoes. Indoor resting mosquitoes were collected in the sentinel and randomly selected houses for 15 min using sucking tubes and flashlights.

Pyrethrum space-spray collections. After the hand catches were conducted, any remaining resting mosquitoes were collected by the pyrethrum space-spray method (WHO, 1975). Before spraying, all the eaves, windows, doors and other exit points were closed and cloth sheets were spread on the floor. Pyrethrum (0.2% in kerosene) was sprayed using a pressurized hand sprayer. After spraying, the room was kept closed for 15 min and the knocked-down mosquitoes were then collected from the floor sheet with forceps and placed in Petri-dishes lined with moist cotton.

Perceived side-effects. The net impregnators and net users were questioned regarding any perceived side-effects of the insecticide-treated mosquito nets.

Results

Susceptibility status of malaria vectors

Insecticide susceptibility tests performed on adult wild-caught females showed that for *An. culicifacies* 83.6% survival with DDT (4% *a.i.*), but there was complete susceptibility to malathion (5% *a.i.*) and deltamethrin (0.05% *a.i.*). *Anopheles fluviatilis* was found to be completely susceptible to all three of these insecticides.

Bioassays on nets

Persistence of the insecticidal effect of deposits from the tablet formulation of deltamethrin on treated nets is shown in Fig. 1. *Anopheles fluviatilis* showed 100% mortality after 3-min exposure and a 24-h recovery period, for up to 6 months, but at the seventh month, mortality was 96.7%, whereas *An. culicifacies* showed 100% mortality only for the first 2 months and a decline to 71.9% mortality by the seventh month.

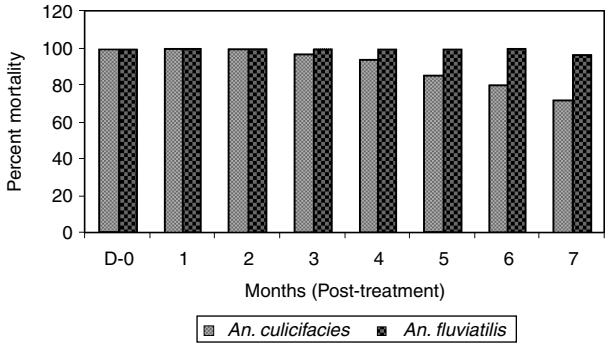

Fig. 1. Bioassays with 3-min exposure to mosquito nets treated with tablet formulation of deltamethrin against *Anopheles culifacies* and *An. fluviatilis*.

Mosquito collections in houses

Table 1 shows the results of collections in four pre-intervention and six post-intervention months in eight rooms in each village by hand catch, pyrethrum space catch, floor sheet and exit traps. The total of the four collections of *An. culicifacies*, other anopheline species and *Culex* spp. were similar in the three villages in the pre-intervention period. However, in that period the collections of *An. fluviatilis* were much higher in Birkera, which later received treated nets, than in the other two villages. After intervention, the collections of *An. culicifacies* and *An. fluviatilis* were much less in the village with treated nets than in the other two villages. However, no such effects were found with treated nets on other anophelines or on *Culex*. The clearest contrast in the numbers of the vector species caught in the different villages before and after treating the nets was seen in November 2002 compared with December 2002 to January 2003. In the last 3 months of data collection few mosquitoes were caught in any of the villages, except that any effect of the DDT spraying in March 2003 in the village without nets was scarcely perceptible by then.

Table 1. Total numbers of mosquitoes caught by resting catches, pyrethrum spray catches, exit traps and on floor sheets, in houses with treated nets, untreated nets and no nets during the pre-intervention and intervention periods. Mosquitoes were collected from four regularly used sentinel rooms and four randomly selected rooms every month. Numbers in brackets are the mean catch per room. Number of collections = 32 pre-intervention phase and 48 intervention phase.

	No. of mosquitoes collected from houses with		
Species	Treated nets	Untreated nets	No nets
Pre-intervention (August 2002–November 2002)			
Anopheles culicifacies	303 (9.5)	326 (10.2)	284 (8.9)
An. fluviatilis	275 (8.6)	15 (0.5)	40 (1.2)
Other anophelines	213 (6.6)	215 (6.7)	192 (6.0)
Culex spp.	92 (2.9)	86 (2.7)	80 (2.5)
Intervention phase (December 2002–May 2003)			
An. culicifacies	20 (0.4)	77 (1.6)	93 (1.9)
An. fluviatilis	2 (0.04)	30 (0.6)	32 (0.7)
Other anophelines	67 (1.4)	64 (1.3)	87 (1.8)
Culex spp.	108 (2.2)	109 (2.3)	124 (2.6)

Immediate and delayed mortality

Table 2 shows that the treated nets caused considerable anopheline mortality during the night, as indicated by the fact that a relatively large proportion of all mosquitoes collected were dead, and virtually all of the live-caught anopheline mosquitoes were dead within 24 h. Among the *Culex* spp. there was an even greater amount of delayed mortality.

Feeding success

Table 3 shows the proportions of blood-fed mosquitoes caught by all four methods. Both treated and untreated nets gave some protection to their users from being bitten by anophelines. The treated nets gave more protection against the vector species than the physical barrier due to untreated nets. In the case of *Culex* spp., the untreated nets gave little or no protection, but the treated nets were more effective.

Excito-repellent action

Table 4 shows data on the exiting rate from rooms with treated and untreated nets, based on catches in the exit traps as a fraction of all catches. In the case of *An. culicifacies*, other anophelines and *Culex* spp. there was evidence of an excito-repellent effect of the deltamethrin.

Perceived side-effects

The net users were questioned about the presence of side-effects due to insecticide and reported none. The net impregnators complained, however, of irritation on their faces and hands, but none of these effects were serious enough to require medical treatment and the effects subsided after thorough washing.

Discussion

Untreated mosquito nets provide some protection against mosquitoes and malaria, provided that the nets are intact (Bradley *et al.*, 1986; Lines *et al.*, 1987; Curtis *et al.*, 1996; Mwangi *et al.*, 2003). Pyrethroid treatment much improves

Table 2. Overnight and delayed mortality of mosquitoes in houses with treated nets (TN), untreated nets (UN) and no nets (NN) during the first 6 months post-intervention (December 2002–May 2003). Numbers in brackets are the percentage of total number of mosquitoes collected.

Species	Houses	Total no. mosquitoes collected dead and alive*	Dead when collected (%)†	Delayed mortality after 24 h (%)‡
Anopheles culicifacies	TN	20	16 (80.0)	4 (20.0)
	UN	70	0	1 (1.4)
	NN	81	0	1 (1.2)
An. fluviatilis	TN	2	1 (50.0)	1 (50.0)
	UN	28	0	5 (17.8)
	NN	14	0	2 (14.3)
Other anophelines	TN	64	43 (67.2)	20 (31.2)
	UN	34	0	1 (2.9)
	NN	47	0	0
Culex spp.	TN	101	13 (12.9)	57 (56.4)
	UN	69	0	0
	NN	112	0	0

*Mosquitoes collected by hand catch of resting mosquitoes, on floor sheets and in exit traps.
†Dead mosquitoes on floor sheets and in exit traps.
‡Delayed mortality observed in mosquitoes collected by hand catch and exit traps.

protection by preventing mosquitoes from biting through nets, killing them before they find holes in torn nets and by having a community wide 'mass effect' on the vector population when there is high community coverage (Lines *et al*., 1987; Lindsay *et al*., 1989; Curtis *et al*., 1996; Maxwell *et al*., 2003). The present trial re-confirms the greater protective effect of treated over untreated nets against *An. culicifacies* and also against nuisance *Culex* spp. (Table 3), protection from which is important as it encourages high net usage. In the present trial the treated nets killed considerable numbers of mosquitoes (Table 2), which suggests that they may have lead to a 'mass effect' on the village mosquito populations. This may have been at least part of the reason for the observed lower catches of the two vectors in the village with treated nets (Table 1). However, distinguishing a mass effect from insecticidal, deterrent and excito-repellent effects in rooms with treated nets would require sampling in sentinel rooms with and without treated nets in villages with high usage of treated nets (Maxwell *et al*., 2003), and preferably also matching bloodmeals in mosquitoes with that of the blood of sleepers in the rooms where the mosquitoes were caught (Soremekun *et al*., 2004). In the present trial there was no reduction in the catches of non-vector anophelines, by contrast to the vector species (Table 1), which is similar to the occurrence of a mass effect in the vector *An. minimus*, but not in the non-vector anophelines, as reported by Jana-Kara *et al*. (1995) in Assam, India. In Turkey, bednets treated with tablet deltamethrin (K-O TAB®) did not reduce the mean density of *An. sacharovi* in the intervention areas compared with the control areas, although reduction in malaria in the former areas was significant (Alten *et al*., 2003). Thus, in this case, reduction in malaria was due to personal protection of net users without a 'bonus' of a mass effect. The increase in proportions caught in exit traps (Table 4) indicates an excito-repellent effect of deltamethrin-treated nets, as found with nets treated with alphacypermethrin by Maxwell *et al*. (2003) and Soremekun *et al*. (2004).

Table 3. Feeding success of mosquitoes collected from houses with treated nets (TN), untreated nets (UN) and no nets (NN) during the intervention phase (December 02–May 03). Numbers in brackets are the percentage of total number of mosquitoes observed.

Species	Houses	Total mosquitoes observed*	No. of mosquitoes fed (%)
Anopheles culicifacies	TN	20	2 (10.0)
	UN	77	25 (32.5)
	NN	93	72 (77.4)
An. fluviatilis	TN	2	0 (0.0)
	UN	30	6 (20.0)
	NN	32	25 (78.1)
Other anophelines	TN	67	10 (14.9)
	UN	64	9 (17.1)
	NN	82	63 (76.8)
Culex spp.	TN	108	29 (27.2)
	UN	109	73 (67.0)
	NN	129	96 (74.4)

*Mosquitoes collected by floor sheet collections, pyrethrum space-spray, exit traps and hand catches of indoor resting mosquitoes.

A number of field trials have evaluated nets treated with different synthetic pyrethroids (Maxwell *et al*., 1999) or different formulations of the same pyrethroid (Jawara *et al*., 2001; Maxwell *et al*., 2003). The present field trial on the efficacy of a tablet formulation of deltamethrin

Table 4. The possible excito-repellent effect of treated nets. Numbers of mosquitoes caught in exit traps compared to total catches in houses with treated nets (TN) and untreated nets (UN).

Species	Houses	Total catch	No. in exit trap	Excito-repellent rate
Anopheles culicifacies	TN	20	6	0.30
	UN	77	8	0.10
An. fluviatilis	TN	2	0	0.00
	UN	30	4	0.13
Other anophelines	TN	67	21	0.31
	UN	64	7	0.11
Culex spp.	TN	108	34	0.31
	UN	109	17	0.15

against malaria vectors showed generally comparable results with a similar trial undertaken on the flowable (SC) formulation of deltamethrin in the same area (Yadav *et al*., 2001). However, in the latter study bioassays showed 100% mortality of *An. culicifacies* on nets 6 months after their treatment with the SC formulation, which was not achieved in the present study (Fig. 1). In Sundargarh district, malaria transmission is perennial and malaria morbidity is relatively high in the young age groups (Sharma *et al*., 2004a). In the present study area, the peak of malaria incidence coincides with the peak vector density of *An. fluviatilis* associated with its high entomological inoculation rate, whereas *An. culicifacies* plays only a secondary role in the intermediate and low transmission seasons (Sharma *et al*., 2004b). Both these vector species are reported to have sibling species complexes. Among the sibling species of *An. fluviatilis*, species S (98.0%) and T (2.0%), and of *An. culicifacies*, species B (28.0%) and C (72.0%) have been reported from Sundargarh district (Nanda *et al*., 2000). The bioassay results on nets in domestic use (Fig. 1) showed continued high mortality of *An. fluviatilis* for the 7 months during which these tests continued. It is widely believed that nets need to be re-treated every 6 months, but in fact high insecticidal activity has been found for much longer periods of domestic use (Maxwell *et al*., 2003). The present data suggest that good results would be achieved against *An. fluviatilis* with annual re-treatment just before the peak transmission season.

The use of one tablet per net treated may be preferable in some circumstances to measuring out liquid insecticide concentrate to make dilutions for treating batches of nets. However, where there are nets of different sizes, each will absorb the same dose per unit area from such a mixture, whereas the use of a standard tablet per net will lead to a higher dose per unit area on smaller nets than on larger ones.

Acknowledgements

The authors are grateful to M/S Aventis Crop Science India Ltd for a free supply of K-O TAB® tablets and partly funding the project. The excellent technical support provided by the staff of the MRC field station is gratefully acknowledged. The community in the study villages deserves our special thanks for their co-operation and participation in the trial. The study was conducted under the Integrated Disease Vector Control Project, funded by the Indian Council of Medical Research (ICMR) and Ministry of Health and Family Welfare, Government of India.

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Accepted 26 May 2005
First published online 28 July 2005