Original Article

Deltamethrin Impregnated Bed Nets in a Malaria Control Program in Chabahar, Southeast Baluchistan, I.R. Iran

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ABSTRACT

A field trial was carried out in the Chabahar District of Sistan and Baluchistan Province, southeastern Iran on the efficacy of mosquito nets treated with a suspension formulation of deltamethrin (K-O THRIN®) against malaria vectors. Treated nets were used in three villages, and in the two control villages, one used untreated nets and the other used indoor spraying with deltamethrin (WP 10%), without nets. Treatment of polyester mosquito net with a target dose of 25 mg/m^2 active ingredient, carried out in mid-April 2005. Bioassays repeated on domestically used nets over 7 months showed persistence of almost 100% mortality of *Anopheles stephensi* over this period. Adult mosquitoes were collected by pyrethroid space spray catch of living quarters and stables, cattle bait and pit shelter catches. Overall, *An. culicifacies* Giles s.l. predominated (49.8%), followed by *An. stephensi* Liston s.l.(36.9%), *An. pulcherrimus* Theobald (7.3 %), *An. dthali* Patton (5.7%) and *An. fluviatilis* James (0.3%). Acknowledge, Attitude and Practice (KAP) study showed that about 97% of respondents in communities agreed that they would like to participate in malaria control activities such as personal protection using impregnation bed net. Therefore, in planning for future large scale trials, comparison of new compounds and formulations such as tablets and long lasting insecticides impregnated bed net is recommended.

Keywords: Malaria control, Deltamethrin, Iran

INTRODUCTION

Despite several years campaigning against malaria, about 107 countries and territories have areas at risk of malaria transmission. Some 3.2 billion people live in areas at risk of malaria transmission. An estimated 350-500 million clinical malaria episodes occur annually; most of these are caused by *Plasmodium falciparum* and *P. vivax* (WHO 2005). Malaria is still the most important parasitic and vector-borne disease in the country. It is one of the main health problems in the south-east provinces. Expect cases from southeastern districts of Iran and a few endemic foci of the other parts of the country, the other clinical cases of the diseases are imported (Vatandoost et al. 2006). A total of 18700 malaria cases were reported in 2005, among which only 70% were Iranian, with the remaining coming from abroad. According to current reports, 90% of cases have been reported from three provinces in the southeast: Hormozgan, Kerman and Sistan and Baluchestan. In these three provinces, chloroquine and sulfadoxine was still the drug of choice for malaria treatment (Center for Diseases Control and Prevention, unpublished data). Malaria transmission in this endemic area occurs through the year, most of the malaria cases were observed from May to November almost in the age groups of 5-25 yr

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old (Moosa kazemi et al. 2006). *An. culicifacies* and *An. stephensi* are responsible for the transmission of 60-70% of the malaria in Iran (Zaim 1998).

Synthetic pyrethroids such as, 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. 2001). 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). There is much emphasis on social marketing of insecticide-treated nets and some doubt whether nets provided free-of-charge will be looked after by householders (Maxwell et al. 2006).

In Iran, trials of insecticide-treated nets carried out during the 1995 to 2000 in southern provinces have shown promising results against malaria transmitted by *An. stephensi* Liston (Moosa-Kazemi et al. 2000, Rassi et al. 2002), and *An. culicifacies* (Zaim et al. 1998). Insecticide-treated mosquito nets are now one of the operational strategies recommended by the World Health Organization and Center for diseases control and prevention of Iranian Ministry of Health.

Because of the importance of the Chabahar County not only for malaria transmission but also for its economic situation in Iran, it was decided to evaluate the effectiveness of deltamethrin impregnated bed nets on *Anopheles* mosquitoes for the first time in this area during 2005. The aim was to obtain new data which would be valuable to develop programmes for improving community health and future planning of malaria control in this endemic area of malaria, southeastern Iran.

MATERIALS AND METHODS

A field trial study was performed from Mar. to Nov. 2005 in Sistan and Baluchistan Province. This study took place in five randomly selected rural villages in Zar-Abad rural district (57°14'N, 26°19'E), 220-250 km far from the city of Chabahar, Iran. It is situated at an altitude of 17 m in a plain area. In 2005 the maximum and minimum mean monthly temperatures were 32.5 °C and 6 °C in July and Jan., respectively. The total annual rainfall was 220.5 mm, the minimum of 3.4 mm in July and maximum of 65 mm in April. The mean annual relative humidity was 74%. The main economic activity of the people is husbandry of cow, goats and fishing.

On the basis of available epidemiological data from the Chabahar Health Centre, some villages were short-listed and preliminary rapid fever and entomological surveys were carried out. On this basis, five villages with 1092 houses and 4464 population were selected and randomly three villages assigned as the trial village to receive treated nets with deltamethrin SC 0.05 (AgrEvo, Marseille, France), one village with 892 inhabitants to receive untreated nets and one control villages with a total of 881 were also selected randomly that received no nets and carried out indoor residual spraying with deltamethrin (WP 10%) in 2005.

An open-ended questionnaire was applied to obtain knowledge and belief of people about malaria transmission, before the residual insecticides application began. To validate and adjust the questionnaire to the understanding of the population, a preliminary and piloted version was conducted to 20 mothers of a village in the study area and no problem was identified. The questionnaire included two sections: the first part 20 questions were designed to obtain information on address of the families; family size, age, sex, marital status, level of education, occupation, sign and symptom of malaria, malaria episodes and background treatment. In the second part, 25 questions, including their knowledge about most important diseases in the area, route of malaria transmission, control measure, their beliefs and practices, including medication and use of health services, perceived effect of indoor residual insecticides, the prefer spraying frequency, the use of other prevention methods such as bed nets and repellents were included. To avoid bias questionnaire were completed without the presence of health workers (Table 1).

The mosquito nets were of polyester poly filament fiber, 100-denier strength, white in color, 156-mesh hole size (12×13 holes/in^2) in family (130×180×150cm) and X-Family sizes (190× 180×150) cm (WHO, 1997). Individual bed net were soaked in a known volume of water, wrung out and allowed to drip so that the excess water in the bucket was meseared and the different between this and the original volume was the amount of the water retained by the net. This was divided by the surface area of the net to obtain the retention capacity in milliliters per square meter. The concentration of insecticide on the absorptive capacity and target dose (25 mg ai/m^2) of the bed net, were prepared. After impregnations, the bed nets were laid on plastic sheet in the shade to partially dry and then hung on the wire to dry completely before they were distributed to the people. A total of 983 bed net, 423 family and 560 X-Family sizes, were impregnated and distributed free of charge in the area. The net impregnators and net users were questioned regarding to any perceived side-effects of the insecticide-treated mosquito nets.

Before starting 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 in the mid of April 2005. The size and number of nets distributed to each household were recorded. The study population was requested not to wash the nets during the study and this was complied with very well. 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. Small pieces of netting of a similar type were then sewn to the net for bioassay analysis.

The persistence of the insecticide on nets in regular use was determined by contact bioassay using a standard procedure (WHO 1980). Because a laboratory colony of *An. culicifacies* was not available, we used a susceptible laboratory colony of *An. stephensi* for detecting changes in the persistence of insecticides on the bed net. The contact bioassay test was performed immediately after net treatment and thereafter at monthly intervals. The mosquitoes used were 12 to 24- h old, unfed, laboratory-reared female *An. stephensi* and they were exposed for 3 min in group of 5, into the adult WHO bioassay test conical. The control female mosquitoes were exposed to untreated mosquitoes netting. At the end of the exposure time the mosquitoes were transferred to clean cups where they were maintained on 10% sucrose solution. The number of mosquitoes that were knocked down by the end of the exposure period and the mortality rate after 24 h holding was recorded. Five replicates of tests, each with 10 mosquitoes, were exposed on each side of the net.

The insecticide susceptibility status of wild caught adult *An. culicifacies* and *An. stephensi* against DDT (4%) and deltamethrin (0.05%) was determined by standard 30, 60, 90 and 120 min exposures to insecticide-impregnated papers, followed by 24-h keeping in holding tube (WHO 1975,1981).

Pyrethroum space spray mosquitoes collection were conducted between 05.00 and 08.00 h monthly in eight fixed animal and human shelter randomly on each villages from April through November, 2005 (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. Attempts were also made to catch mosquitoes resting outdoors using mouth suction tube. All collected mosquitoes were identified to species and classified based on abdominal condition (Shahgudian 1960).

RESULTS

The population, history of malaria and number of questionnaire applied in the treated, untreated and control villages, during the years 2004-2005 are presented in Table 1.

A total of 1092 people were interviewed; 1216 from treated and 186,198 from untreated and control villages, respectively, comprising 4464 inhabitants in five villages. A total of 1984 questionnaires were obtained. The age groups in three areas almost was the same and 32% of the population was under 15 yr old, 35% between 15-29 yr old, 16% between 30-44 and 18% were 45 yr and older, family size was 4 to 5 years, the majority of head families were active in husbandry and fishing (30%). The rest domestic activities (19%), government workers (14%), self employed (13%) and unemployed (8%). Almost over two- third (70%) of the population were illiterate and the others were employed schooling.

Most of the mothers were house keeper, the others were engaged as husbandry and fishing activities.

Eighty seven percent of interweaves (n= 950) indicated that they have malaria experience, 56% (n= 611) indicated that at least one member of their family had malaria during the pervious years. In this study, the most known sign of malaria were the same and comprises fever 52%, chills 25%, headache 10.5%, dizziness 5%, others 1.7%. The majority of interweaves believed it was transmitted by mosquitoes bites (98%) and the rest stated that is was transmitted by stale food, non potable water and unhygienic surrounding. Significantly residual spraying was mach more common stated in treated and control villages interweaves (odds ratios= 2.41, X^2= 42.79, df= 1, *P*= 0.0001). When they were asked about the time of use, 25% indicated in evening and night and75% using at night in both areas, 63% to 67% of the interweaves stated they rest outdoor (in the yard) whereas, of the them stated indoor places through warm season. A total of 99% responded that thy brought at least one product for treatment against malaria.

Fig. 1 presents the results of the 24 h mortality of 12 to 24 h old, unfed laboratory reared female *An. stephensi* when exposed to mosquitoes net impregnated with deltamethrin for 3 min. Mortality rates were considered more stable.

The susceptibility test on *An. stephensi* and *An. culicifacies* wild strain is presented in Table 2. Insecticide susceptibility tests showed that for *An. stephensi* 39.4% survival with DDT (4% a.i.) after one hour exposure, but there was complete susceptibility to deltamethrin (0.05% a.i.). *An. culicifacies* was completely susceptible to all two of these insecticides.

From April to November 2005, a total of 9843 female *Anoheles* mosquitoes were collected by pyrethrum space spray catches from indoor places of living quarters and stables (Table 3). Overall, *An. culicifacies* Giles s.l predominated (49.8%), followed by *An. stephensi* Liston s.l. (36.9%), *An. pulcherrimus* Theobald (7.3 %), *An. dthali* Patton (5.7%) and *An. fluviatilis* James (0.3%). In the control village, where no residual spraying has been used the density of mosquitoes was higher than other places and *An. stephensi* Liston was predominated (48.8%) followed by *An. culicifacies* Giles s.l. (38.5%), *An. fluviatilis* James (6.7%), *An. pulcherrimus* Theobald (5.57%) and *An. dthali* Patton (0.22%). In the untreated area *An. culicifacies* Giles s.l was predominated (60.5%) followed by *An. stephensi* Liston (25.9%), *An. fluviatilis* James (7.8%), *An. pulcherrimus* Theobald (1.75%) and *An. dthali* Patton (0.45%). The composition of anopheline species was different in control and untreated villages as this area. In treated area *An. culicifacies* Giles s.l was predominated (51.6%) followed by *An. stephensi* Liston (34.5%), *An. fluviatilis* James (7.4%), *An. pulcherrimus* Theobald (6.3 %) and *An. dthali* Patton (0.245%).

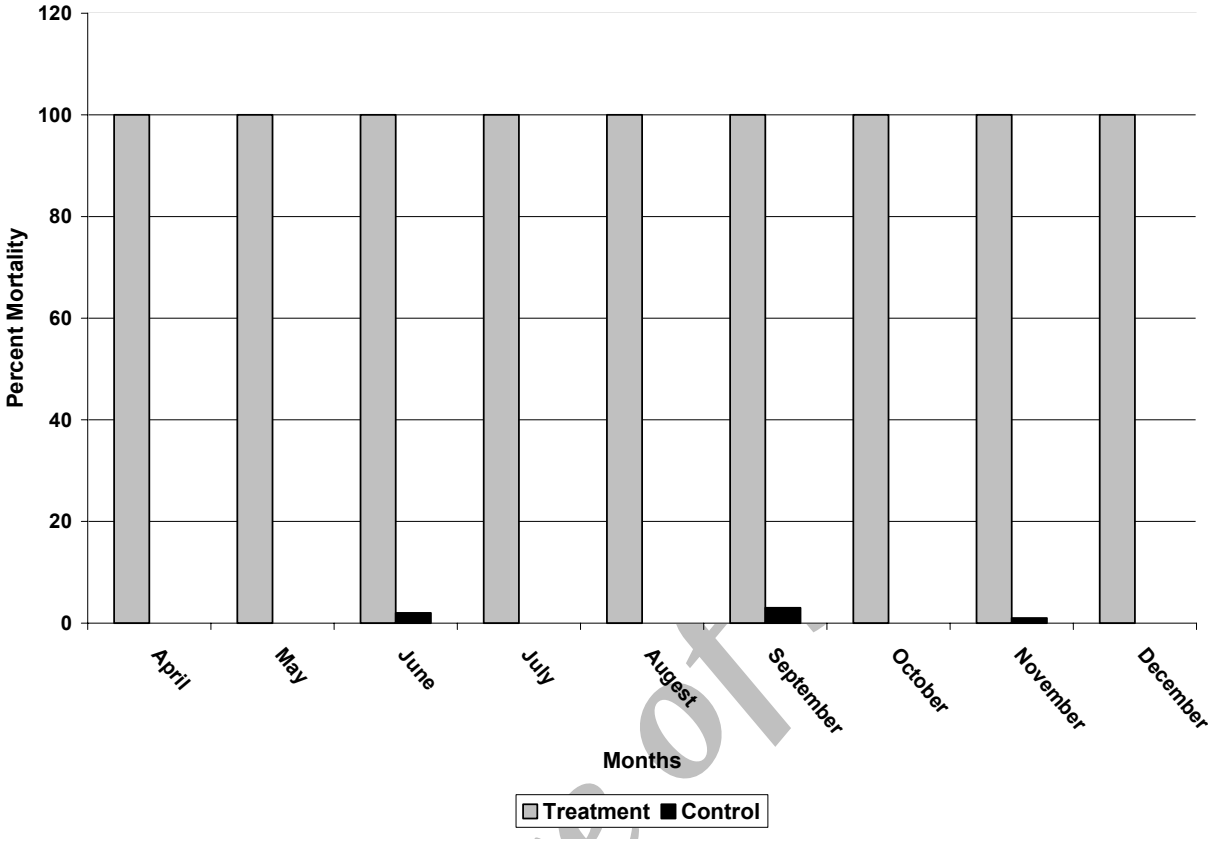

Fig. 1. Bioassy test with 3-minutes exposure to mosquitoes net treated with deltamethrin against *Anopheles stephensi* in Zar Abad, Chabahar, Sistan and Baluchistan Province, Iran 2005

Table 1. Inhabitants, history of malaria cases, annual parasite incidence (API) per 1000 population and number of questionnaires applied per villages in Chabahar, Sistan and Baluchestan Province, 2004-2005

Area	2004			2005			Families interview	Questionnaires applied
	Pop.	No. cases	API	Pop.	No. cases	API		
Treatment	2736	114	41.6	2798	104	37.2	608	1216
Untreated	859	46	57.5	892	43	48.8	186	372
Control	877	43	49.4	881	44	50.0	198	396

Table 2. Susceptibility test on *An. stephensi* and *An. culicifacies* in Chabahar County, Southeastern Iran (2004-2005)*

Insecticides	Species	Control	Mortality percent after exposure to insecticide impregnated paper and 24 h post exposure holding			
			30	60	90	120
DDT	*An. stephensi*	0 (76)	14.5 (89)	29.4 (85)	46.8 (78)	100 (98)
	An. culicifacies	0 (82)	62.4 (98)	100 (94)	91.3 (112)	100 (108)
Deltamethrin	*An. stephensi*	0 (84)	100 (109)	100 (104)	100 (108)	100 (96)
	An. culicifacies	0 (96)	100 (122)	100 (92)	100 (110)	100 (98)

*Figures in parenthesis are number of mosquitoes tested

Table 3. Total number, the monthly mean indoor resting density, of mosquitoes catches by pyrethrum space spray and hand collection from various sites of study villages in Zar Abad District, Sistan and Baluchestan Province 2004-2005.

Species	Treated			Untreated			Control			Total	
	No.	mean	%	No.	mean	%	No.	mean	%	No.	%
An. culicifacies	1524	261.1	51.6	1989	248.6	60.5	1389	173.6	38.5	4902	49.8
An. stephensi	1017	127.1	34.5	854	106.7	25.9	1761	220.2	48.8	3632	36.9
An. fluviatilis	219	27.3	7.4	256	32	7.8	244	30.5	6.7	719	7.3
An. pulcherrimus	187	23.3	6.3	173	21.6	1.75	201	25.1	5.57	561	5.7
An. dthali	6	0.75	0.2	15	1.87	0.45	8	1	0.22	29	0.3

DISCUSSION

This is the first report of the evaluation of deltamethrin impregnated bed net to malaria control in Chabahar County. Previous study in Iran was carried out in Dishmook, Bandar Abbas and Ghassreghand Counties. The use of protective and preventive measure against malaria is related to family income and consequently to the capability of purchasing explicitly stated by the participant in our study. In spite of the limitation mosquito nets are a familiar household item and 83% of the mothers' families in Chabahar stated they used bed net and 13% and 17% some times user in this area.

Bed net has been used in different parts of the world with varying results. 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 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, Maxwell et al. 2006). The density of the vectors was reduced when impregnated bed net were introduced (Zaim et al. 1998, Moosa Kazemi et al. 2000, Rassi et al. 2002). Our study showed that the use of deltamethrin impregnated bed net can provide any protection from the bite of *Anopheles* species and subsequently interrupted the malaria transmission but does not provide complete protection from the diseases.

Although, there is a reduction in the density of female *An. culicifacies* in indoor places of treated compared with the untreated also *An. stephensi* compared with the control villages but the results showed no significant different between these area ($P< 0.05$).

Syntethic pyrethroid combines several advantages for use on the net. This compound as having excito-repellency, quick-acting, and are effective in the small quantities on the bed nets. Deltamethrin and lambdacyhalothrin are reported to persist on treated net for one year, even with one or two washing (WHO 1980). Our study showed that *An. stephensi* and *An. culicifacies* to be completely susceptible to DDT and deltamethrin insecticides. Mortality rates were considered more stable when *An. stephensi* exposed to mosquitoes net impregnated with deltamethrin for 3 min. The bioassay results on nets in domestic use (Fig. 1) showed continued high mortality of this species 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. 2006). The present data suggest that good results would be achieved against anopheline main vectors with annual retreatment just before the peak transmission season.

In the present trial the treated nets reduced considerable numbers of mosquitoes (Table 3), 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 three vectors in the village with treated nets. However, distinguishing a mass effect from insecticidal, deterrent and excito-repellent effects in rooms with treated nets would require sampling in rooms with and without treated nets in villages with high usage of treated nets (Maxwell et al. 2003). 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.

A number of field trials have evaluated nets treated with different synthetic pyrethroids (Maxwell et al. 2006) or different formulations of the same pyrethroid (WHO 1980, 2002). The present field trial on the efficacy of a suspension concentration formulation of deltamethrin against malaria vectors showed generally comparable results with a similar trial undertaken 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 achieved on *An. stephensi* in the present study (Fig. 1). In Chabahar County, malaria transmission is perennial and malaria morbidity is relatively high in the young age groups (Center for Diseases Control and Prevention, unpublished data). Two distinct peaks of malaria transmission occur in the Chabahar County: one in April-May and the other in September- October. In the present study area, the peak of malaria incidence coincides with the peak vectors density of *An. culicifacies* and *An. stephensi* as the main vectors, whereas *An. fluviatilis* plays only a secondary role in the transmission. However *An. culicifacies*, identified as species A of the complex (Zaim and Javadian 1991), but species B (28.0%) and C (72.0%) have been reported from Sundargarh District (Nanda et al. 2000).

Conclusively, at the present time, with regard to failure effects of vaccine against malaria and the compliance of the residents with the operational residual spraying, personal protection is an effective and sustainable means of preventing of the diseases. The use of standard tablet for net treated may be better than liquid or suspension therefore in planning future large scale trials, comparison of new compounds and formulations such as tablets and long lasting of insecticides recommended.

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