Effect of insecticide-treated bednets for malaria control in Southeast Anatolia-Turkey

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ABSTRACT: Deltamethrin is one of the most effective insecticides for vector control, already widely used for bednet impregnation to control malaria. To evaluate the efficacy of deltamethrin-impregnated bednets in malaria control and in reducing the biting nuisance caused by *Anopheles sacharovi,* field trials were carried out in an endemic area of malaria in the surrounding rural settlements of ª anliurfa City, SE Anatolia, Turkey. Preliminary studies commenced in August 1999 with pre-intervention house-to-house surveys to identify villages outside of ª anliurfa City with high malaria incidence, to collect socio-economic, epidemiological and entomological data, and to determine physical properties of the study areas. An intervention field trial promoting the use of K-OTAB (deltamethrin-tablet formulation) impregnated bednets by local inhabitants of four villages was performed between July 2000 and July 2001. Its aim was to examine the monthly and annual efficacy of such bednets in controlling malaria and to compare the effect of impregnated bednets (IB) with non-impregnated bednets (NIB). The experimental design consisted of four villages. Gedik was selected as the intervention area using IBs, Orgulu served as the control area, and in Persiverek and Sandi NIBs were implemented. All 1,406 inhabitants of the 146 households were recruited for the study. Results showed significant ($P<0.05$) reduction in malaria incidence in Gedik from 8.29% in the pre-treatment year to 1.57% in the post-treatment year. In contrast, malaria incidence slightly increased in Orgulu from 6.55% to 7.58%. Similar results were obtained from the other two villages where NIBs were used; malaria incidence rates increased from 2.16% to 6.77% (Persiverek) and from 1.9% to 9.8% (Sandi). Entomological surveys, employing different techniques, were carried out randomly at selected collection sites within the intervention and control settlements every month from June 2000 to June 2001 to determine the fluctuation of seasonal population sizes and compare the monthly density of malaria vectors between intervention and control areas. ***Journal of Vector Ecology* 28(1): 97-107. 2003.**

Keyword Index: Malaria, *Anopheles sacharovi,* bednet, deltamethrin.

INTRODUCTION

Malaria was the most important vector-borne disease in Turkey until 1965, but following the establishment of the malaria eradication program in the mid 1950s, the incidence of malaria gradually decreased. In view of the success in controlling the disease, the national health authorities decided to reduce the investments made on the control activities and malaria services were thus considerably reduced (Ramsdale and Haas 1978). An agricultural development program initiated in the mid 1970s in the Cukurova Plain caused a substantial migration of workers from the eastern areas where malaria at that time was more prevalent. This population movement, together with other factors such as industrial expansion and widespread vector resistance against insecticides, resulted in a serious epidemic of malaria in 1977 in the provinces of Adana, Hatay and Icel, with 101,867 reported cases. Presently, malaria is still one of the most important health problems in Turkey. Malaria has become hyper-endemic in the southeastern part of Turkey due to the GAP irrigation network projects. The most recent outbreak involved approximately 11,000 clinically reported cases with many more unrecorded in different parts of the country (Turkish Ministry of Health Statistics). Two-thirds of these cases were recorded in the ª anliurfa province.

Thirteen *Anopheles* species have been recorded in Turkey (Ramsdale et al. 2001). Among these species, *Anopheles sacharovi* is the most important vector of malaria, with *An. superpictus, An. claviger* and *An. hyrcanus* playing minor roles. Historically, three indigenous parasite species have been reported in Turkey: *Plasmodium malaria, P. falciparum* and *P. vivax.* Currently, although occasional imported cases of *P. malaria* and *P. falciparum* are observed, all indigenous cases of malaria are *P. vivax*.

There is an increasing trend to use insecticide-impregnated bednets for major interventions in malaria control programs. Pyrethroid-impregnated bednets are advocated by the World Health Organization for personal protection against malaria vectors. To avoid the need for periodic re-treatment, it would be advantageous for nets to be able to retain insecticidal efficacy for years and withstand repeated washing (N’Guessan et al. 2001). During the past decade, pyrethroid-treated mosquito nets have become established as an important defense against malaria transmission (Curtis et al. 1990, Carnevale et al. 1991, Choi et al. 1995, Lengeler et al. 1998). Large-scale epidemiological field trials involving community use of pyrethroid-impregnated bednets have demonstrated major benefits in reducing malaria morbidity and mortality (Snow et al. 1988, Alonso et al. 1991, 1993, Dapeng et al. 1994, Jaenson et al. 1994, Stich et al. 1994, D’Alessandro et al. 1995a, 1995b, Binka et al. 1996, Nevill et al. 1996, Goodman et al. 1999, Habluetzel et al. 1999, Rashed et al. 2000, Abdulla et al. 2001, Armstrong-Schellenberg et al. 2001, Minja et al. 2001, Guyatt and Snow 2002).

Although conventional malaria control programs using residual insecticide spraying have been conducted by the Turkish Ministry of Health in collaboration with WHO and several private companies, apart from case findings and treatment studies there is little information on feasible means of controlling *Anopheles* species and malaria in Turkey. The only reported attempt was a trial of insecticide house-spraying and fogging of related areas in SE Anatolia. This brought a transient reduction but rapid re-invasion of the vector from unsprayed areas. However, where appropriate, this method of control is rapidly being complemented globally by the use of insecticide impregnated bednets. This is the preferred technology being used in the WHO Roll Back Malaria program. Although it has joined the RBM initiative, Turkey has little experience in the use of insecticide-impregnated bednets.

This study describes intervention trials to prevent malaria through the use of K-OTAB impregnated bednets in rural settlements surrounding ª anliurfa City in Turkey.

MATERIALS AND METHODS

Study area

The study was carried out during August 1999-August 2001 in ª anliurfa province (37° 09’ N; 38° 47’ E), SE Anatolia-Turkey. The province, located along the border of Syria in the south, comprises 18,500 km^2 of the western part of the south-eastern Anatolia region. Four villages for the trials (Gedik, 37°45’N; Orgulu, 39°19’E; Persiverek, 37°49’N; Sandi, 39°43’E) were identified as highly endemic with active cases of malaria ranging from 1.90% to 8.29% with a total population of 1,406 (Table 1). The chosen settlements are very difficult geographic and climatic conditions, situated within mountainous and volcanic areas at an altitude of about 1500 m ranging from 1300 m to 1650 m. The people in the area are mostly farmers, with many of the men working as manual laborers in cotton fields situated in the Harran Plain, Diyarbakir and Batman. Many small-scale cattle farms are worked in the area and some people work as laborers in nearby small factories, but agricultural production is the primary source of income. Rice, corn, tomatoes and grain are the most important agricultural products in this area.

Study design, impregnation and distribution of bednets

Between August 1999 and June 2000, three pre-intervention surveys were conducted in the chosen operational areas to determine the incidence rates for the pre-intervention year (May 1999 to May 2000). These consisted of three large scale questionnaires conducted with 5,000 inhabitants, using optical forms. Statistical procedures were carried out on the physical characteristics of settlements, socio-economic levels of populations living in the experimental areas, migration/

Table 1. Settlements and type and number of distributed bednets.

Settlements	Population	No. of households	Bednet types			
			Single	Double	Family	X-family
Gedik (IB)	567	22	71	100	75	12
Persiverek (NIB)	185	72	24	31	16	6
Sandi (NIB)	211	19	20	30	19	4
Örgülü (control)	443	33				
TOTAL	1406	146	115	161	110	22

IB: intervention with treated nets.NIB: intervention with untreated nets. Control: no intervention.
Family: 3 persons. X-family : 4-5 persons.

immigration rates of areas and the ratio of malaria cases, to compare the settlements with each other and decide on the main intervention and control areas. In April 2000, the questionnaires were conducted in the chosen settlements with 1,406 inhabitants to determine incidence rates, sleeping patterns of households and to determine the number of bednets required for distribution to householders. The last questionnaire was conducted to determine annual incidences in the settlements prior to the intervention, which commenced in June 2000.

Polyester bednets (mesh size 156/cm^2; 100 denier) made by Siamdutch Mosquito Netting Co., Thailand, were impregnated with K-OTAB at 25 mg a.i./m^2 following the method described by Schreck and Self (1985) and distributed to households in the intervention and control settlements (Table 1). The number distributed to each household was proportional to the size and structure of the families. Gedik was selected as the intervention area using IBs, Örgülü as the untreated control and the two others, Persiverek and Sandi, as intervention using NIB. All the 1,406 inhabitants of the 146 households were recruited for the study.

After the intervention, follow-up questionnaires were conducted every 45 days between August 2000 and August 2001 to determine the fluctuation of malaria incidence and to compare settlements to each other. All the bednets distributed to the IB areas were re-impregnated every six months by our field team. HPLC tests were carried out after intervention at three-month intervals (just after intervention, at three months and at six months) between June 2000 and January 2001. Fifteen treated bednets were used for HPLC tests each time. A total of 90 bednet pieces were cut from distributed bednets (from top, bottom, right side, left side, front and behind the bednet) and were analyzed by Normal Phase HPLC (after extraction with 90/10 Isooctane/THF) on a Chromospher Si column. Mobil phase was 97/3 Isooctane/1.4 Dioxan and UV detection at 236 nm.

Entomological sampling

Entomological sampling was done using the collection methods of light traps, conventional aspirators, and human and animal traps for mosquito collection. These were carried out in ten randomly selected houses in the intervention and control settlements every month between December 1999 and June 2001. To determine the population size and seasonal fluctuation of *Anopheles* species in the intervention and control settlements, houses and barns were numbered and used as sampling sites. While determining the sampling sites, criteria such as distance to the breeding habitats of mosquito larvae or the settlement center were rounded off. For the sampling of the exophilic species, light traps were maintained according to the standard used by Southwood (1966). A total of 20 light traps were used for sampling. Light traps were operated between the hours 19:00-07:00 at each sampling station every month.

A sampling method dependent on time was used to determine the fluctuations of the population size of endophilic mosquito species within the houses and barns. Samples were taken with aspirators that had a 50 cm long plastic tubing connected to a 75 cm glass pipe. Samples were taken every month by two men, allowing 10 minutes for each house and barn.

Statistical analysis

The significance of possible sources of error (treatment and date) was assessed by Kruskal-Wallis one-way analysis of variance (ANOVA) according to Zar (1996).

RESULTS

Monthly fluctuations in malaria incidence

Malaria incidence rates in the four study villages were as follows: 4.44 % for Persiverek, 3.48% for Sandi, 6.47% for Gedik and 3.56% for Örgülü two months after

Table 2. The monthly fluctuations of malaria incidence in the villages.

	Incidence (%)						
Villages	Jun 00*	Aug 00	Oct 00	Dec 00	Feb 01	Apr 01	June 01
Örgülü (Control)	6.6	3.6	4.5	1.2	0	0	0.7
Persiverek (NIB)	2.2	4.4	2.4	1.0	0	0	0
Sandi (NIB)	1.9	3.5	3.6	2.5	0	0	1.4
Gedik (IB)	8.3	6.5	6.4	0	0	3.3	0

*Annual incidence rates determined before intervention (May 1999 to June 2000) in the villages.
IB: intervention with treated nets. NIB: intervention with untreated nets. Control: no intervention.

Table 3. Statistical comparison of malaria incidence in the villages during consecutive monthly periods.

	Gedik (IB)						Örgülü (Control)					
	Aug-00	Oct-00	Dec-00	Feb-01	Apr-01	Jun-01	Aug-00	Oct-00	Dec-00	Feb-01	Apr-01	Jun-01
Aug-00		0.969						0.32				
Oct-00	*P*>0.05		0.028				*P*>0.05		0			
Dec-00		*P*<0.05		1				*P*<0.05		0.148		
Feb-01			*P*>0.05		0.262				*P*>0.05		1	
Apr-01				*P*>0.05		0.262				P>0.05		0.395
Jun-01					*P*>0.05						P>0.05	

	Persiverek (NIB)						Sandi (NIB)					
	Aug-00	Oct-00	Dec-00	Feb-01	Apr-01	Jun-01	Aug-00	Oct-00	Dec-00	Feb-01	Apr-01	Jun-01
Aug-00		0.088						0.979				
Oct-00	*P*>0.05		0.246				*P*>0.05		0.45			
Dec-00		*P*>0.05		0.355				*P*>0.05		0.056		
Feb-01			*P*>0.05		1				*P*>0.05		1	
Apr-01				*P*>0.05		1				P>0.05		0.268
Jun-01					*P*>0.05						P>0.05	

Test statistics : Kruskal-Wallis

*T*he differences (Gedik, 0.038=*P*<0.05; Orgulu, 0.00=*P*<0.05; Persiverek, 0.00=*P*<0.05; Sandi, 0.010=*P*<0.05) observed in malaria incidence rates for all months are significant. IB: intervention with treated nets. NIB: intervention with untreated nets. Control: no intervention.

the end of intervention. These rates gradually declined in the following months (Table 2). Although a small decrease was observed in the malaria incidence in the village of Gedik between the months of August and October, a rapid decrease was seen after October. The same situation also appeared in the villages of Persiverek, Sandi and in the Örgülü village of the control group. In general, a decline in the malaria incidence was observed in the towns of Persiverek and Sandi where only untreated bednets were distributed and in the town of Gedik where treated bednets were distributed. When all months were evaluated, the fall in the malaria incidences was statistically significant (*P*<0.05) (Table 3).

When the malaria incidence rates of the regions were analyzed and all months compared, the differences were significant (*P*<0.05). When the malaria incidence rates of Persiverek and Sandi were compared for consecutive monthly periods, there was no significant difference. However, a significant difference was observed in the malaria incidence rates in the village of Gedik between October and December (*P*<0.05) (Table 3). The same situation was observed in Örgülü as a significant difference in the malaria incidence rates between October and December (*P*<0.05), but not a significant difference among the consecutive months.

Annual changes in malaria incidence: Impact of insecticide-impregnated bednets on reduction of malaria incidence

Treated bednets were distributed to the village of

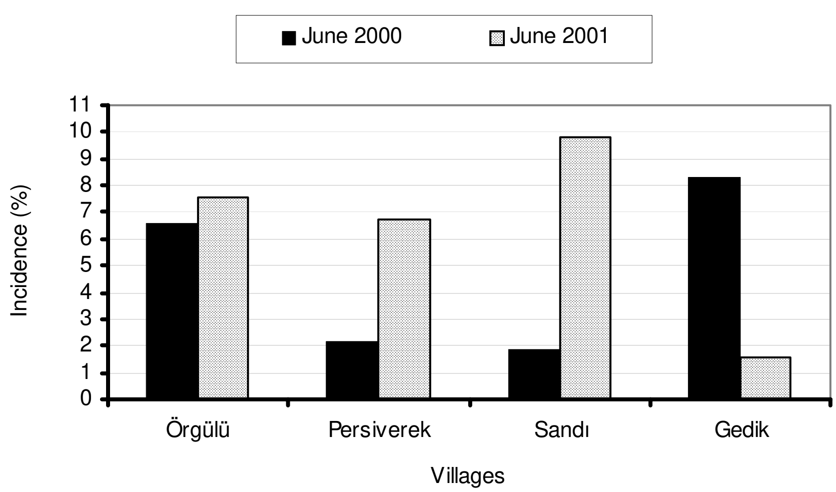

Figure 1. The annual changes of malaria incidence in villages. Gedik: intervention with treated nets. Persiverek and Sandi: intervention with untreated nets. Örgülü : control - no intervention.

Table 4. The statistical comparison of malaria incidence in villages for 2000-2001(Kruskal-Wallis).

Villages	Differences	
Örgülü (Control)	0.552	*P*>0.05
Persiverek (NIB)	0.031	*P*<0.05
Sandi (NIB)	0	*P*<0.05
Gedik (IB)	0	*P*<0.05

IB: intervention with treated nets.
NIB: intervention with untreated nets.
Control: no intervention.

Gedik, untreated bednets were distributed to the villages of Persiverek and Sandi, and the village of Örgülü served as the untreated control. At the beginning, 5,000 people were interviewed, 1,406 of whom were enrolled in the intervention study. When the villages were analyzed individually for a period of one year, the incidences at the beginning (June 2000) were 2.2% (4/185) for Persiverek (NIB), 1.9% (4/211) for Sandi (NIB), 8.3% (47/567) for Gedik (IB) and 6.6% (29/443) for Örgülü (control). At the end of the intervention (June 2001-post intervention period) the respective incidence rates were 6.8%, 9.8%, 1.6% and 7.6%, respectively (Figure 1).

The malaria incidence showed an increase despite the bednets (NIB) in the villages of both Persiverek and Sandi, but showed a significant decrease in the village of Gedik where treated bednets (IB) were utilized. This demonstrated that the malaria incidence surprisingly increased from 2.2 and 1.9% to 6.77 and 9.81%, respectively in villages where only untreated bednets (NIB) were utilized. These unexpected results obtained from NIB trials were caused by some circumstances beyond our control that will be assessed and addressed in the Discussion.

The statistical comparison of incidence for 2000-2001 was analyzed (Table 4). We found that, in all three of the villages at the end of the one-year period, the decrease or increase in the incidence was significant ($P<0.05$). Based on the annual change observed in the malaria incidence rates, it is apparent that treated bednets (IB) had a reducing effect on the malaria incidence.

The village of Örgülü was the untreated control group that was compared with the village of Gedik where treated bednets (IB) were distributed. To determine the influence of the insecticide, untreated bednets (NIB) were distributed to the villages of Persiverek and Sandi. At the beginning of the study, a significant difference ($P<0.05$) was detected in the malaria incidence between the villages of Örgülü and Persiverek + Sandi and again between the villages of Örgülü and Gedik ($P<0.05$) (Table 5). The Örgülü village differed significantly from intervention villages for the incidence of malaria at the beginning. At the end of the study, there was no significant difference between the Persiverek and Sandi, where only untreated bednets were distributed, and Örgülü. In contrast, the difference determined between the villages of Persiverek and Sandi (NIB) and the village of Gedik (IB) were significant. This significant difference

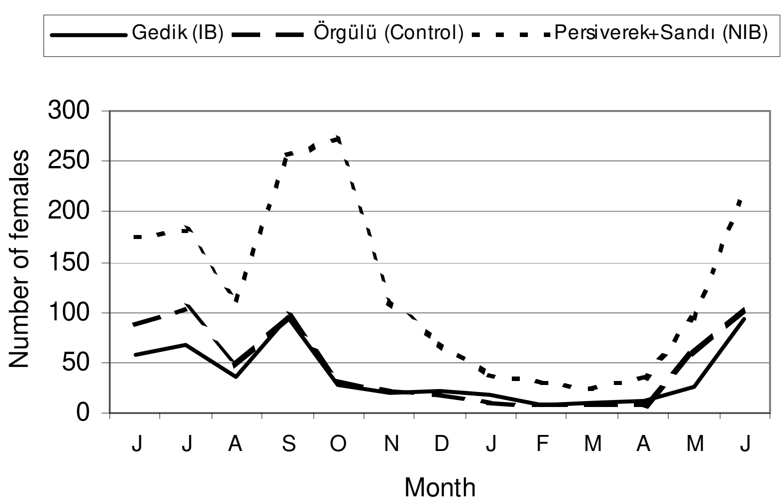

Figure 2. Seasonal population fluctuations of *An. sacharovi.*

was determined at the end of the study and was not present at the end of the study. This indicates the importance of using bednets, whether IB or NIB.

Entomological results

A total of 4,446 mosquitoes was collected in the study area between June 2000 and May 2001, belonging to *Anopheles sacharovi* and *An. superpictus.* The former is the main malaria vector in Turkey and was found to be the pre-dominant species. Overall, 2,668 *An. sacharovi* were captured, representing 60% of the total number of mosquitoes collected. *An. superpictus* were only collected in the NIB area (Persiverek and Sandi villages). Figure 2 shows the seasonal population fluctuations of female *An. sacharovi* collected in the IB (Gedik), control (Örgülü) and NIB (Persiverek and Sandi) areas during the survey season. In general, there were no significant differences, not only in the total population size of each month, but also in the indoor and outdoor population between Gedik and Örgülü. Indoor population sizes of both areas for all the months were slightly more than that of their outdoor populations. In contrast, both indoor and outdoor populations and total population of the NIB areas were determined to be significantly different than either IB or control ($P<0.05$). In the NIB areas, the total indoor population size was determined to be three times higher than the total outdoor population size, especially during September. There were no significant differences in the monthly population size of *An. sacharovi* between intervention (IB and NIB) and control areas, but the population size of NIB was found to be significantly different than that of the IB and control areas especially during the summer season ($P<0.05$) (Figure 2). Two peaks were identified, the first occurring in July and the second in September in the IB and control areas. These peaks were recorded later, namely in September and October, in the NIB area. While the highest population size was found in July in the control area, it was observed in September and October in the IB and NIB areas, respectively.

HPLC test results

The results of HPLC analysis made on bednets used during the intervention are shown in Table 6. Just after impregnation, the dosage on some of the treated nets was too high and coverage was uneven. This unevenness was probably due to the relative difficulty of ensuring a uniform suspension in the final dilution. Nine results were omitted from the mean calculations since they appeared anomalous with the other replicates. Although analysis of nets after aging for three months or six months showed little, if any, decline in the level of dosage (1 to 2.1 mg / m^2), consecutive analyses showed that there was an adequate quantity of active ingredient on the surface of the net fibres to provide a satisfactory result during a six-month period. Additionally, no significant differences were found in the levels of active ingredient of pieces cut from various parts of the bednets, even three and six months later ($F_{.05}$ (3.116) F=0.18). This result demonstrated that all parts of the bednets used in the study were sufficiently impregnated by insecticide.

As discussssed in similar studies, the effectiveness of the treated nets can be reduced by using an inadequate dosage, and from loss or reduced dosage of insecticide due to the washing of treated nets. The treatment efficacy is considered to be more dependent on the amount of washing than on the time after the treatment (Curtis et al. 1990, Anonymous 1997). The insecticidal effect on a treated net at any given time may be checked with bioassays to provide guidance for re-treatment of nets in operational use. Generally, nets that are freshly treated with the recommended dose of an insecticide are expected to yield almost 100% mortality of target anophelines. After a period of use, and in particular after washing, the mortality tends to decline. From the operational context, the efficacy of the pyrethroids in treated bednets is expected to last 6-12 months depending on the insecticide, but less with nets washed frequently (Anonymous 1997). In our study, health education messages were relayed to the study population to encourage the use of bednets and every opportunity was seized to encourage the population to comply with the correct and frequent use of the bednets to attain prevention. The interviewers also drew attention of the households to the washing effect on nets. It was found that less than 1% of impregnated bednets were washed at least once by users living in the intervention area (Gedik) throughout the study period, thus the removal of residual insecticide from impregnated nets was generally negligible (Table 6). In conclusion, it was determined that the K-OTAB formulation had a long-lasting effect on the bednets under natural conditions in the study area.

DISCUSSION

The efficacy and community-effectiveness of insecticide-impregnated bednets in preventing morbidity and mortality from malaria in several malaria endemic countries such as Africa, Asia and South America has been frequently demonstrated (Alonso et al. 1993, Dolan et al. 1993, Jaenson et al. 1994, Stich et al. 1994, D'Alessandro et al. 1995a, 1995b, Binka et al. 1996, Nevill et al. 1996, Rowland et al. 1996, Lengeler et al. 1998, Goodman et al. 1999, Armstrong-Schellenberg et al. 2001, Guyatt and Snow 2002). Our study shows that use of

Table 5. The statistical comparison of the malaria incidence in villages for the years 2000 and 2001.

Village	2000 Örgülü	Pers+Sandi	Gedik
Örgülü (control)		0.01	0
Pers+Sandi (NIB)	*P*>0.05		0.145
Gedik (IB)	*P*>0.05	*P*<0.05	

Village	2001 Örgülü	Pers+Sandi	Gedik
Örgülü (control)		0.675	0
Pers+Sandi (NIB)	*P*<0.05		0
Gedik (IB)	*P*>0.05	*P*>0.05	

IB: intervention with treated nets. NIB: intervention with untreated nets. Control: no intervention.

deltamethrin-impregnated bednets can provide significant personal protection from the bites of mosquitoes and subsequently reduce the risk of malaria infection in the rural areas of [a] anliurfa, Turkey but does not totally eradicate it.

The results are based on data collected from four villages endemic with malaria, a small number for a field trial, although more than 1,000 people were enrolled. However, the results illustrate the impact of this technique in reducing malaria incidence. The most important outcome measure in determining the effectiveness of impregnated bednets is the incidence rate of the study population and settlements.

Many physical characteristics of the houses and environment and some characteristics of householders of the study population were found to be highly significant in increasing the incidence for malaria before the intervention, according to findings determined from questionnaires. For example, if most of the houses in a malarious area have wooden ceiling material, adobe construction material, or water collection tanks or wells, the incidence of malaria may be higher. Some additional parameters, such as lower education and economic levels, a greater number of people living in the same house, watching television or sleeping in the garden or on the roof during hot weather while not using any protective materials such as bednets, and high immigration rates could all be considered as factors causing the relatively high levels of malaria incidence. Results concerning high incidence obtained from the villages where untreated bednets were distributed (Persiverek and Sandi) confirmed the above related statements. Detailed information on these risk factors will be presented elsewhere.

There was no strong evidence in this study, as in similar studies (Dolan et al. 1993, Stich et al. 1994), that the use of pryethroid-impregnated bednets had an impact on reducing the mean total density of *An. sacharovi* in the intervention areas compared with the control areas. The mean density was similar in the intervention and control areas during the 2000-2001 transmission season (Figure 2). It might be expected that the insecticide absorbed by the bednets would have resulted in a lower mean mosquito density in the intervention area compared with the control area. However, dead insects, including *An. sacharovi*, were found in the mornings on and around the treated bednets during survey team visits to households in the intervention settlements. This indicates a possible impact on the reduction of the overall density of the insect population, including mosquitoes.

Human behavior plays an important role in influencing mosquito behavior, as the insects tend to extract blood meals from human subjects, thus transmitting the disease. For example, people choose their sleeping place arbitrarily in the hot summer months. From early June to the end of September they either sleep indoors or in the courtyards using traditional wooden beds, depending on the weather, and many would stay awake and watch television in the courtyards. Because *An. sacharovi* is mainly endohilic and endo-exophagic (Hadjinicolau and Betzios 1973, Ramsdale and

Table 6. The results of HPLC analysis of the bednets used during intervention.

Position of piece	Dosage (mg/m^2) After impregnation	3 months	6 months
Top	24.48*	23.99	23.31
Bottom	23.12	22.99	22.00
Right side	27.10	26.09	24.02
Left side	23.67	22.17	22.00
Front	24.01	23.61	22.89
Behind	25.56	22.88	22.11

* Average value of all pieces cutted from fifteen bednets

Haas 1978, Gilles and Warrell 1993), it is difficult to estimate the reduction in the mosquito density. This situation is different from other study areas where inhabitants do not have electricity or television and go to sleep and wake up early mainly indoors. These difficulties are confirmed by the results of previously published studies that found no significant impact of insecticide-impregnated bednets in reducing the vector population that transmits malaria. Lindsay and McAndless (1978) found that the use of insecticide treated jackets did not have a significant impact in reducing the survival of mosquitoes in intervention villages although insecticide-treated jackets provided protection for people by reducing malaria incidence.

Our study has shown a considerable and consistent reduction of malaria in the intervention areas following the introduction of deltamethrin-impregnated bednets compared with the control area where no bednets were distributed. Although *An. sacharovi* were abundant in the intervention area, the results of the study suggest that their ability to transmit malaria was greatly reduced because of the treated bednets. However, besides the known benefits of insecticide-treated bednets, the operational problems encountered in re-treating bednets with insecticide are often cited as an impediment to wide-scale implementation (Guyatt and Snow 2002). Recent operational studies have shown that the most significant barrier to effective implementation was the ability to re-treat nets. Experimental studies on the introduction of fees for re-treatment have demonstrated marked reductions in re-treatment rates (Winch et al. 1997, Kachur et al. 1999). In most operational projects with a cost recovery element, only 5% to 30% of nets are re-treated (Guyatt and Snow 2002). It is very clear that the low re-treatment rate of nets is a major cause of concern for those advocating insecticide-treated nets (Guyatt and Snow 2002). Because of this, recent investigations into the protective effects against malaria were overtaken by studies focusing on the benefits of untreated bednets. The evidence for a protective effect of untreated nets against malaria has been presented in many studies alongside an analysis of how well untreated nets would need to work in order to compete with treated nets within a cost-effectiveness framework (Guyatt and Snow 2002). Many of the published cross-sectional studies have demonstrated reduced levels of malaria infection in children sleeping under untreated bednets (Genton et al. 1994, D'Alessandro et al. 1995a, Abdulla et al. 2001, Clarke et al. 2001). These span more than a decade and consistently show a protective effect of untreated nets against malaria parasitemia by as much as 51% (Guyatt and Snow 2002). Choi et al. (1995) showed that insecticide-treated bednets had a 50% lower protective efficacy against malaria re-infection when the control used untreated nets (24%) than when insecticide-treated bednets were compared with no nets (50%). This suggests that the net alone could be responsible for half of the protective efficacy of treated bednets. Similar patterns have been observed for mild malaria in the recent meta-analysis by Guyatt and Snow (2002). Very convincing evidence came from different studies where untreated nets use in settlements had a significant effect on mortality in children that was independent of insecticide impregnation. Under operational conditions, untreated nets could have a protective efficacy against all causes of mortality of 19% in children between 1 month and 4 years old (Armstrong-Schellenberg 2001). The combined evidence given above indicates an important protective effect of untreated bednets.

The results we obtained were considerably different from the expected results and results found in other investigations. In the two villages where untreated bednets were distributed, malaria incidence increased after a period of one year (Figure 1). We considered four basic reasons for these unexpected results.

First, as seen in Table 2, the malaria incidence increased in the above-mentioned villages during August-October 2002. The main reason for this was the large number of immigrant workers in the cotton fields. The immigration rate was 57% during this period, the largest in the past 10 years (Anonymous 2001), and 95 % of this population came from Batman and Diyarbakir cities where malaria is endemic. The population (752 persons) that was taken under control by distributing untreated bednets at the beginning of the intervention increased to 1,180 persons in a 5-month period. The existence of malaria parasites in 72.9% of the people that immigrated to the experimental area was determined by the Health Ministry specialists. In addition to this, the population in this area is very conservative, traditionally selecting their employees from their relatives. For this reason, a large portion of workers stayed with the families under the control (NIB) group. After a short amount of time, the number of bednets that were distributed to the people living in the villages became inadequate and extra bednets for the new population could not be distributed. Likewise, the close relationship between the population under control (villagers) and not under control (workers) caused high increases in the malaria incidence.

Second, at the beginning of the implementation, extensive health training took place in the villages that received treated and untreated bednets. While being trained, the villagers received information about malaria and mosquitoes as well as the usage of the bednets. However, this training was not repeated for the immigrants because of geographical conditions and long distances. As a result, after a short period, even people who received the training were influenced by the behavior of workers and started to not use the bednets during dinner time, TV watching and even sleeping when the *An. sacharovi* population was high (20:00-22:00 hrs).

Third, in the villages that received untreated bednets, it became known that the bednets were not treated with insecticide, so the results were not effective. The questionnaires showed that a lot of families did not use the untreated bednets for this reason. Finally, the implementation was carried out for one year. This period may have been too short to make a healthy comparison between the effects of treated and untreated bednets. For these reasons, the controlled experiments became uncontrolled and unexpected and statistically meaningless data were sometimes obtained.

An. sacharovi larvae are difficult to control because they occur in numerous small puddles in rice fields, ponds, etc. in mountainous areas. The adults enter houses and animal sheds to rest and feed with feeding taking place during the night when most people are sleeping. Thus bednets, especially if made more effective by pyrethroid impregnation, should offer effective protection against malaria transmission in ª anliurfa. The nets should also reduce the vectorial capacity of malaria vectors.

The high efficacy of insecticide-impregnated bednets in reducing malaria incidence as found in our study together with similar results emerging from other trials will have important policy implications. For example, it should lead the way for less dependence on the current policy of selective residual insecticide spraying and could lead to the eventual adoption of a new policy encouraging the use of insecticide-impregnated bednets to control the disease in malaria foci. Moreover, the extensive experience, gained in the trials using the same technique for cutaneous leishmaniasis, could prove helpful in effectively implementing this strategy in the urban sites of ª anliurfa where CL incidence is always higher than malarious rural sites, due to the more suitable geographical and socio-economical conditions.

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