Evaluation of deltamethrin-impregnated bed nets and curtains for control of zoonotic cutaneous leishmaniasis in a hyperendemic area of Iran.


(1) Department of medical entomology and vector control, School of public health and Institute of public health research, Tehran University of medical sciences, P.O.Box 6446-14155, Tehran, Iran. Tél. : (+98-21) 88973901, 66461334 (Extension 2114), Fax : (+98-21) 66462267. E-mail : yaghoobia@sina.tums.ac.ir
(2) Isfahan training and health Research center, Institute of public health research, P. O. Box 334-81465, Isfahan, Iran.
(3) Disease management center, Ministry of health and medical education, Tehran, Iran.
(4) Department of epidemiology and biostatistics, School of public health & Institute of public health research, Tehran University of medical sciences P.O. Box 6446-14155, Tehran, Iran.


Résumé : Évaluation des rideaux et moustiquaires imprégnés de deltaméthrine dans la lutte contre la leishmaniose cutanée zoonotique d’une zone hyperendémique d’Iran.

Cette étude a été conduite dans le district rural de Borkhar (Ispahan, Iran), de mars 2003 à mars 2004. L’efficacité des moustiquaires (IBs) et des rideaux (ICs) à mailles de polymère de 156 trous au pouce carré (25 trous/cm²), imprégnés de deltaméthrine, était comparée à celle des moustiquaires (NIBs) et des rideaux (NICs) non imprégnés, en vue de contrôler la leishmaniose cutanée zoonotique (ZCL) dans cette zone. Les moustiquaires et rideaux imprégnés par 25 mg a.i/m² ont été distribués dans 168 maisons d’un des villages et ceux qui n’étaient pas imprégnés dans le même nombre de maisons d’un autre village. Dans le troisième village, le même nombre de maisons a été utilisé comme témoin.

Des messages d’éducation pour la santé ont été diffusés pour s’assurer que la population se conforme à l’utilisation correcte des moustiquaires et des rideaux dans les deux zones d’intervention. La surveillance épidémiologique portait sur l’utilisation de pièges à papier collant qui ont été placés tous les 15 jours dans dix maisons de chaque village pour capturer les phlébotomes, tant à l’intérieur qu’à l’extérieur, afin de mesurer l’impact des moustiquaires et des rideaux imprégnés sur la densité de Phlebotomus papatasi. Des tests de sensibilité à la deltaméthrine et des tests biologiques ont porté sur cette espèce selon la méthode OMS standard.

Les données ont été fournies lors des visites effectuées maison par maison, une fois par saison, et tous les habitants des maisons sélectionnées dans chaque village ont été examinés. Les résultats ont montré que les moustiquaires et les rideaux imprégnés de deltaméthrine procurent, sans équivoque, une protection personnelle contre les piqûres de phlébotomes et permettent d’interrompre la transmission de la leishmaniose muqueuse zoonotique dans les villages où ils ont été utilisés. Par contre, les moustiquaires et rideaux non imprégnés ne procurent pas de protection contre la maladie. Il n’y avait pas de différence significative de densité mensuelle de P. papatasi tant à l’intérieur qu’à l’extérieur des maisons dans les trois villages (P > 0,05). Les tests de sensibilité ont montré que, dans cette zone, cette espèce était sensible à la deltaméthrine sur le terrain. Les tests biologiques ont confirmé que les moustiquaires et les rideaux restaient efficaces plus de trois mois après leur imprégnation à la deltaméthrine et constituaient un excellent moyen de protection individuelle. On peut donc recommander une utilisation très large des moustiquaires et rideaux imprégnés de deltaméthrine pour le contrôle de la leishmaniose cutanée zoonotique dans d’autres foyers iraniens, tant en zone hyperendémique que mésoendémique.

Summary: In the study carried out in the rural district of Borkhar (Ispahan, Iran) from March 2003 to March 2004 efficacy of deltamethrin impregnated bed nets (IBs) and curtains (ICs), polyester mesh size 156 holes/ inch², (25 holes/cm²) were compared to that of non-impregnated bed nets (NIBs) and curtains (NICs), in relation to zoonotic cutaneous leishmaniasis (ZCL) control. Deltamethrin impregnated bed nets and curtains at 25 mg a.i./m² were distributed among 168 households in one of the villages and NIBs plus NICs among the same number of households in another village. In the third village similar numbers of households were used as control.

Health education messages were disseminated to ensure the population’s compliance with the proper use of bed nets and curtains in the two intervention areas. Entomological surveys using sticky paper traps were carried out in ten households in each village for the collection of sand flies from indoors and outdoors, every two weeks to assess the impact of insecticide impregnated bed nets and curtains on the density of Phlebotomus papatasi. Deltamethrin susceptibility tests and also bioassay tests were carried out on this species by WHO standard method.
Case findings were done by house-to-house visits once a season and all the inhabitants of the selected households in each village were examined. The results showed that IBs and ICs can provide a definite personal protection against sand fly bites and interrupted the transmission of ZCL in the intervention village. NIBs and NICs did not provide any protection against the disease. There was no significant difference in monthly density of P. papatasi in indoors and outdoors among the villages (P > 0.05). Susceptibility tests showed that this species was susceptible to deltamethrin in the field population in the area. Bioassays confirmed that the treated nets with deltamethrin remain effective for more than three months and was an excellent mean of individual protection. It is recommended that IBs and ICs can be used widely in the control of ZCL in other similar foci such as hyperendemic and mesoendemic areas of Iran.

Introduction

Zoonotic Cutaneous Leishmaniasis (ZCL) is a serious and increasing public health problem in Iran, hyperendemic in rural areas to the north and east of the city of Isfahan, among the foothills of the Karkas mountains in central Iran. In this focus, intensive transmission occurs, so that many indigenous residents acquire the disease before the age of five (4, 17). Almost 60% of all reported cases of the disease in this country come from this focus (12, 15, 17). The great gerbil, Rhombomys opimus (Rodentia: Gerbillinae) is the main animal reservoir. The red tailed Jird, Meriones libycus (Rodentia: Gerbillinae) has also been found infected. The main vector to humans and gerbils is Phlebotomus (Phlebotomus) papatasi, but P. (Paraphlebotomus) caucasicus, P. (Para.) mongolensis and P. (Synphlebotomus) ansarii also act as vectors among gerbils and jirds.

In the last two decades, the increasing number of ZCL cases in the traditional foci and their spread to new foci in west and southwest near central desert (Badrood, Abouzaid-abad, Ardestan, Abarkhoul) and the south of the country (Neiriz, Estahban, Lar, Darab, Jahrom, Kharameh and Sarvestan), has been of concern to the health authorities (26). Human prevalence of the disease and the incidence of new cases remain high despite passive case detection and treatment by well trained staff in local clinics and public health services in Isfahan. House spraying with DDT and application of DDT powder in rodent burrows have failed to control ZCL in this area (13, 20).

It should be mentioned that other methods of intervention, such as autoclaved Leishmania major (ALM) vaccine with BCG did not provide protection against ZCL and the safety and efficacy of this vaccine with other adjuvant is under consideration at present (11).

Bed nets and curtains impregnated with pyrethroid insecticides have been used successfully against anopheline mosquitoes (2, 5, 7, 10) and against phlebotomine sand flies (1, 8, 9). Moreover, pyrethroid impregnated bed nets have been used with a relative success against anthropoponic cutaneous leishmaniasis in Syria (22) and Iran (16) Afghanistan (19), Turkey (3) but no study has been carried out on the effectiveness of this method to control ZCL in our country yet. This encouraged us for evaluation of the same method plus impregnated curtains to control ZCL in a hyperendemic focus of Iran.

The selected villages are a part of endemic focus of Isfahan which is situated on the plain. The soil of the area is generally a little salty, and sandy in some parts (14). However, wheat, barley, cotton, vines, beetroot, pistachio, alfalfa, Indian corn, clover and summer crops are cultivated. The area has a desert climate, very hot in summer and cold in winter. In 2003, the maximum and minimum mean monthly temperature were 39.1°C and -2.8°C in July and December, respectively. The total annual rainfall in 2003 was 124 mm, minimum of 5.4 mm in March and maximum of 49.6 mm in January among rainy months. The minimum mean monthly relative humidity was 12% (August and September) and the maximum was 89% (January).

The endemicity of ZCL is so high that almost 70% of the population contract the disease before the age of ten (14, 27). The community is familiar with the disease by its local name (Copheh). The parasite has been identified as Leishmania major zymodeme MON 26 (30). The major reservoir host is R. opimus and its average infection rate is 55% (28). The main vector to man is P. papatasi and natural promastigote infection of this species is 11% in rodent burrows (29).

Impregnation and distribution of bed nets and curtains

Three villages (called Komshecheh, Aliabad-Mollaali, and Habibabad) were selected randomly in the rural district of Borkhar, Isfahan province, central Iran. Then, in each village, 168 households near each other with similar prevalence (2.1-2.7% for lesions and 70.4-81.2% for scars) were recruited to the study. Also according to our previous study in 2002, the incidences of the disease were similar in these villages and were calculated to be 38.5, 38.0 and 37.7 per thousand respectively (Institute of public health research, unpublished data). Besides, treatments were randomly performed in corresponding villages. All households enrolled in Habibabad received impregnated bed nets and curtains (IBs and ICs); Aliabad-Mollaali, non-impregnated bed nets and curtains (NIBs and NICs) and Komshecheh was decided to be the control area. White polyester bed nets and curtains (mesh size 156 holes/inch2, 25 holes/cm2, 75 denier) made by a factory in Tehran were impregnated with deltamethrin SC 5% (K-Othrine); 25 mg a.i./m2 following the method described by WHO, in 2002 (25).

After weighing, the bed nets were soaked and the extra water was squeezed out. Then, the bed net was weighed again to check the quantity of water absorbed. Next, the total surface of the net was divided to find out how much water was absorbed by one square meter of each type. After calculating the amount of the insecticide, the final solution was prepared in a big plastic basin. Water and insecticide were mixed thoroughly using a large glass rod. The net was put in an empty plastic basin and the insecticide solution was added gently, allowing the net to soak for several minutes while turning it constantly.
with a wooden stick. Then the net was lifted and wringed gently. After impregnation, the nets were laid on plastic sheets in the shade to partially dry. They were then hung on a wire in the shade for several hours to dry completely.

The impregnated bed nets were then carefully wrapped in black nylon bags and labeled with the size (single, double and family size) and the date of impregnation. They were then kept in a cool, dry storage place until the time of distribution.

The same method of impregnation (K-Othrine; 25 mg a.i./m²) was used for curtain material. Impregnated bed nets (IBs, single, double and family size) and non-impregnated bed nets (NIBs) were distributed to all the selected households in the intervention area, Habibabad and Aliabad-Mollaali respectively, according to their needs in April 2003 and they were encouraged to use them from the sunset to the sunrise in the next morning. The number of bed nets distributed to each household was proportional to the size and structure of the family (Table I).

Windows of all houses were also covered with impregnated curtains (IC) in Habibabad and non-impregnated curtains (NIC) in Aliabad-Mollaali at the same time.

### Health education

Health education messages were relayed to the study population in the intervention area (Habibabad and Aliabad-Mollaali) to encourage the use of bed nets and curtains. The role of sand flies in transmission of the diseases was explained to the people on several occasions by face to face (women and men) and focus group (women) discussion methods during distribution of bed nets and curtains. The staff drew the attention of the households to the importance of using bed nets and hanging the curtains on the doorways and windows when sleeping to protect themselves and their children from leishmaniasis. Schools were visited and teachers were urged to educate their students in the importance of using bed nets and curtains to protect themselves from sand fly bites. From 7 to 10 households were checked at random during the night each week by two teams to ensure that the bed nets and curtains are used.

No intervention operations were done in the village of Komshecheh, the control area.

### Entomological surveillance

Three houses were chosen in each village and sand flies were collected biweekly from ten fixed sites in each house (bedrooms, stables, storerooms, toilets) using 30 sticky traps (castor oil coated white papers 20×35 cm) from the beginning (end of May) to the end (middle of November) of the active season. The same number of sticky traps was also used for collection of sand flies from outdoors (courtyards). Sticky traps were put before sunset, and collected on the following morning, before sunrise. The sand fly species were determined by professional entomologists.

Deltamethrin susceptibility tests were carried out on the field population of _P. papatasi_ by the WHO standard method (23) during July–August 2003. Sand flies were collected from outdoors by aspirator in Aliabad-Mollaali (NIBs + NICs) and Komshecheh (control) and were transferred to the laboratory. Testing was carried out there, free from any insecticidal contamination. The treated sand flies were held at the temperature between 23°C and 25°C, and a relative humidity about 75%. It should be mentioned that during 1925–58, the area was sprayed with DDT one round annually (7 rounds). Bioassay tests were conducted in July, August and September 2003 after 3, 9 and 12 weeks impregnation of bed nets respectively on fed females of _P. papatasi_ adults collected from Aliabad-Mollaali (NIBs + NICs) and Komshecheh (Control), by WHO standard method (24).

### Studies on human Infection

All 504 households (168, each village) in the study were taken census, data were collected from one of the parents of the household for all household residents; their age and sex, relationship to household head, past and present history of ZCL and details of any current lesions (number, date and place of acquiring). In each case with cutaneous lesions, smears were prepared and examined later in Tehran, after staining with Giemsa. The results of microscopic examinations of active lesions were recorded on the related forms.

### Follow-up of study households

Case findings were done by house-to-house visits once every season during the last weeks of June, September, December 2003 and March 2004, and all the inhabitants of the selected villages were examined in each village. Only new cases of ZCL, the number of patients with new lesions were recorded on each visit. Yearly incidence of the disease was determined separately in each village at the end of March 2004. Persons with scars were excluded when yearly incidence of the disease was calculated. Inclusion criteria were persons with active lesions.

### Statistical analysis

The data were entered in SPSS (11.5) and analysed using SPSS (11.5) and STATA (8.0). Graphs were prepared using Excel. One-sided Fisher’s exact test was used to compare incidence rate of ZCL in each village for two consecutive years. The same test was also used to compare the incidence rate between Habibabad (IBs + ICs) and Komshecheh (control). Besides, the Kruskal-Wallis test was used to compare the density of female _P. papatasi_ in indoors and outdoors in each intervention village compared with the control area.

### Results

Comparison of the incidence rates of ZCL in each village between, before and after intervention showed that there is a significant difference in the village of Habibabad (37.7 versus 0; _P = 0.036_; table II) where the IBs and ICs were used. However, in the village of Aliabad-Mollaali, despite using NIBs and NICs, non-significant differences were observed ( _P > 0.05_). The same results were also obtained in the control

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Table II.

Comparison of incidence (per thousands) of zoonotic cutaneous leishmaniasis in the intervention and control villages, Borkhar rural district, Isfahan, Iran, April 2002–March 2003, April 2003–March 2004.

<table>
<thead>
<tr>
<th>Area</th>
<th>Village</th>
<th>Bed net &amp; curtain type</th>
<th>Population</th>
<th>No with active lesion</th>
<th>Incidence 2004</th>
<th>Incidence 2002 (before intervention)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Habibabad</td>
<td>IBs &amp; ICs</td>
<td>574</td>
<td>0</td>
<td>0</td>
<td>37.7</td>
</tr>
<tr>
<td></td>
<td>Aliabad-Mollaali</td>
<td>NIBs &amp; NICs</td>
<td>627</td>
<td>1</td>
<td>15.2</td>
<td>38</td>
</tr>
<tr>
<td>Control</td>
<td>Komshecheh</td>
<td></td>
<td>776</td>
<td>10</td>
<td>37.2</td>
<td>38.5</td>
</tr>
</tbody>
</table>

Table III.

Age distribution of active lesions in intervention and control areas, Isfahan during the study period, 2003-2004.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Habibabad</th>
<th>Aliabad-Mollaali</th>
<th>Komshecheh (Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
</tr>
<tr>
<td>0-4</td>
<td>0</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>5-9</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>10-14</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>15-19</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>20-24</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>25+</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>All ages</td>
<td>0</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

More than 72% of the inhabitants knew the breeding places of sand flies and method of transmission of the disease to human. In the village of Aliabad-Mollaali, 84.6% of the households used the bed nets regularly and 72.5% of the households put up the bed nets at sleeping time (10.00–12.00 pm), but 26.4% put them up at sunset. 87.5% of the households used the bed nets in rooms while 14.3% was using them in the courtyards. All the households kept the bed nets in room during the day. Almost 67% of the inhabitants knew the breeding places of sand flies and method of transmission of the disease to human.

Density of sand flies

During May-November 2003, 896 adult sand flies (460 from outdoor and 436 from indoor resting places) were collected and identified. The following five species were found in bedrooms, warehouses and privies:

- *P. papatasi* (68.58%),
- *P. caucasicus* (2.98%),
- *P. mongolensis* (0.92%),
- *P. sergenti* (2.98%),
- *S. sintoni* (24.54%).

In outdoors (courtyards), *P. papatasi* (69.13%), *P. caucasicus* (2.61%), *P. mongolensis* (0.22%), *P. sergenti* (6.5%) and *S. sintoni* (27.39%) were collected.

The most common sand fly of indoor and outdoor resting places was *P. papatasi*. This species is largely domestic and represents 68.58% of sand flies from indoors and 69.13% from outdoors. *Phlebotomus papatasi* appeared in April or the last days of May and disappeared in November. There were two peaks in the density curve of *P. papatasi* one in June and the second in August (figures 1 & 2). In general, there was no significant difference in monthly density of female *P. papatasi* in indoors and outdoors among the villages (P > 0.05). No sand fly was found in the area during November to March due to cold weather.

Susceptibility tests

The susceptibility of *P. papatasi* to 0.025% deltamethrin was studied in the field surveys, following the WHO standard methods (table IV).
Table IV.

Results of the bioassays percentage mortality of wild-caught *P. papatasi* females after 3 minutes contact and 24 hours observations with bed nets treated with 25 mg/m² deltamethrin in Borkhar rural district, Isfahan 2003.

<table>
<thead>
<tr>
<th>weeks after impregnation</th>
<th>temp.</th>
<th>% RH</th>
<th>% mortality after 24 hours</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>22-29</td>
<td>75</td>
<td>100 (30)</td>
<td>0 (28)</td>
</tr>
<tr>
<td>9</td>
<td>24-30</td>
<td>76</td>
<td>100 (27)</td>
<td>0 (22)</td>
</tr>
<tr>
<td>12</td>
<td>22-28</td>
<td>76</td>
<td>100 (32)</td>
<td>3.5 (28)</td>
</tr>
</tbody>
</table>

Figures in parenthesis are number of sand flies tested.

In this program, 18 series of susceptibility tests were carried out on 671 fed *P. papatasi*. The mortality rates for 0.025% deltamethrin after 2, 4, 7.5, 15, 30 and 60 minutes of exposure time followed by 24 h recovery time were 100%. It is concluded that *P. papatasi* is susceptible to deltamethrin in the field population in this area.

The bioassays

As shown in table IV, the mortality of *P. papatasi* (wild-caught, fed females) was 100% until the end of week 12. The mortality of control sand flies never exceeds 3.5%. It was impossible to continue this study from week 12 onwards because of the decrease of the density of *P. papatasi* due to rains and cold weather in the area.

Discussion

This is the first report on the effectiveness of deltamethrin impregnated bed nets and curtains to prevent ZCL in a hyperendemic area in Iran. Previous studies were carried out in the control of anthropontic cutaneous leishmaniasis (ACL) in Sudan, Syria, Afghanistan, Iran and Turkey (3, 6, 16, 19, 22). Our evaluation shows that the use of both deltamethrin impregnated bed nets and curtains do not provide any protection against the disease. It should be mentioned that about 7% of the households in each village were lost during part of the follow up visits, but as this phenomenon occurred in all villages it does not affect the comparative results. The results of this study allow large scale field trials in an epidemiologically similar area of Iran and also neighbouring countries.

Although, there is a reduction in the density of female *P. papatasi* in indoors and outdoors in the intervention areas compared with the control area (figures 1 & 2), but the Kruskal-Wallis test shows non-significant differences among different months (P > 0.05).

It should be mentioned that cutaneous leishmaniasis of the study area is zoonotic, presumably, the sand flies may feed on many hosts other than humans. This explains why sand fly populations, particularly those outdoors, do not seem to have been reduced. There was also no strong evidence in similar studies (3, 5, 16, 21, 22), that the use of pyrethroid impregnated bed nets had an impact on reducing the mean total density of female *P. papatasi* or *P. sergenti* between intervention areas compared with the control areas. However, dead insects, including sand flies, were found in the morning on and around the treated bed nets during survey team visits to households in the intervention settlements.

We observed no side effects of the pyrethroid impregnated bed nets and curtains and none was reported among the inter-

views during the follow up study. Although it was impossible to continue bioassay tests in October and onwards in the field surveys due to the cold weather but susceptibility tests and bioassays showed that *P. papatasi* was perfectly susceptible to deltamethrin and the bed nets and curtains treated with this insecticide remained effective for 3 months and more. In a trial for controlling cutaneous leishmaniasis vectors in Colombia, the effectiveness of lambdacyhalothrin on bed nets persisted 4 months (18).

The low cost and simplicity of impregnated bed nets and curtains (approximately 5.5 $ US for a bed net, plus 25 cents per impregnation) means that their use could be sustainable in rural communities of Iran and elsewhere, given that local people recognize the nuisance and dangers of sand flies and other biting flies, and are amenable to the use of treated nets. Moreover, most of these people already have some knowledge of the use and application of insecticide, through their present agricultural activities.

In addition to offer good prospects of local sustainability, IBNs and ICs have the advantage of being portable and they require no specialized equipment or trained personnel. This method interrupted the transmission of the disease in the study area, but after the cessation of using IBNs and ICs, the transmission will continue as before.

At the present time, in the absence of a suitable vaccine, personal protection by IBNs and ICs are an effective and suitable means of preventing and controlling ZCL. We feel that the results of this trial justify the more widespread use of IBNs and ICs in controlling other similar foci such as hyperendemic and mesoendemic areas of ZCL of Iran.

Conclusion

Impregnated bed nets and curtains provided good personal protection against the bites of the main vector, *Phlebotomus papatasi*, and interrupted the transmission of ZCL in the intervention village in the study area. At the present time, with regard to the failure effects of ALM vaccine with BCG against ZCL, personal protection is an effective and sustainable means of preventing and controlling the disease. The implication of the positive results of this trial can lead to less dependence to insecticides in endemic areas. It can direct to adopt a new policy by encouraging the use of IBs and ICs to control the disease in similar high risk parts of Iran during of active seasons of sand flies.

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Références bibliographiques

1. ALEXANDER B, USMA MC, CADENA H, QUESADA BL, SOLAR-TIC Y et al. – Phlebotomine sand flies associated with a focus