J Vector Borne Dis 51, December 2014, pp. 307–312

Field evaluation of phostoxin and zinc phosphide for the control of zoonotic cutaneous leishmaniasis in a hyperendemic area, central Iran

A.A. Akhavan[1, 2], A. Veysi[2] , M.H. Arandian[3], H. Vatandoost[1, 2], M.R. Yaghoobi-Ershadi[2], M. Hosseini[4], H. Abdoli[3], K. Heidari[5], A. Sadjadi[5], R. Fadaei[5], J. Ramazanpour[5], K. Aminian[5], M.R. Shirzadi[6] & R. Jafari[3]

[1]*Institute for Environmental Research (IER),* [2]*Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran;* [3]*Esfahan Health Research Station, National Institute of Health Research, Tehran University of Medical Sciences, Esfahan;* [4]*Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran;* [5]*Esfahan Health Centre, Esfahan University of Medical Sciences, Esfahan;* [6]*Department of Zoonosis, CDC, Ministry of Health and Medical Education, Tehran, Iran*

ABSTRACT

Background & objectives: ZCL is a growing threat in many rural areas of Iran which involves 17 out of 31 provinces. This study was conducted from April to November 2011 for evaluation of the efficacy of phostoxin and zinc phosphide against rodents.

Methods: Rodent control operations were carried out using phostoxin and zinc phosphide. To evaluate the effect of rodent control operation on the main vector density, an entomological survey was carried out. The effects of the operation on the disease incidence were also evaluated.

Results: After intervention, the reduction rate of rodent burrows was 32.68% in the village treated with phostoxin and 58.14% in the village treated with zinc phosphide. The number of rodent holes in the control area showed 6.66-fold increase at the end of the study. The incidence of the disease decreased to 19.23 and 11.40 in areas treated with phostoxin and zinc phosphide, respectively. A total of 4243 adult sandflies were collected and identified. The most common and dominant species was *Phlebotomus papatasi*. In the village treated with phostoxin, the density of *P. papatasi* in outdoors was lower than indoors. Nevertheless, the density of *P. papatasi* in the village treated with zinc phosphide was higher in outdoors.

Interpretation & conclusion: It is concluded that phostoxin is less effective and has low safety in comparison with zinc phosphide, so that this rodenticide can be used only in special situations such as lack or ineffective rodenticides and only in the colonies far from human and animal dwelling places in small scales.

Key words Iran; *Phlebotomus papatasi;* phostoxin; rodent control; zinc phosphide; zoonotic cutaneous leishmaniasis

INTRODUCTION

Zoonotic cutaneous leishmaniasis (ZCL) is a growing threat in many rural areas of Iran which involves 17 out of 31 provinces[1].There are four different epidemiological zones of ZCL in the country and four species of rodents (Gerbillinae) known as the principal animal reservoir hosts in different foci. *Rhombymos opimus* is the main animal reservoir in the northeast and central part of the country. *Meriones libycus* has been found as a principal reservoir host in some parts of central and south of the country. *Tatera indica* is known as the main reservoir host in the southwest of Iran and *Meriones hurrianae* in southeast of the country, neighbouring to Pakistan[2–5]. *Phlebotomus papatasi* is the most prevalent species among *Phlebotomus* genus, and is the only proven vector of ZCL[6–7]. Moreover, *P. caucasicus*, *P. mongolensis* and *P. ansari* also considered as vectors among gerbils and jirds. *Phlebotomus papatasi* is the main and proven vector of *Leishmania major* transmission to man in Turkmenistan, Uzbekistan, Saudi Arabia, Iran, southern Morocco and central Tunisia[8–9]. So far, *L. major* has been isolated and identified from naturally infected *P. papatasi, P. caucasicus*, *R. opimus, M. libycus* and in humans in some endemic areas of Iran[3–5, 10].

Iranian researchers have employed various methods to control ZCL in different parts of the country since 1996. Some control measures such as residual spraying with DDT, spraying powder of DDT in the rodent burrows, poisoning the reservoir hosts and using deltamethrin-impregnated bed nets and curtains have been employed to control ZCL in the country[11–13]. Furthermore, to control the disease, a successful leishmanization has been conducted in Iran in special circumstances. It has been recommended just for military personnel in very high risk areas[14]. One intervention study showed that autoclaved *L. major* (ALM) vaccine with BCG had not been protective against ZCL[15].

In recent years, attempts to control ZCL have been followed by experts in the country. In 1997, through a field trial, rodent burrows were destroyed and baited with zinc phosphide 2.5% in a radius of 500 m from houses once in a month in May, June, July and September. The results showed 12-fold reduction in incidence of ZCL in treated village, compared to the control village at the end of first year and 5-fold at the end of second year of the operation[16]. From 1999 to 2002, in the same intervention area, for evaluation of previous study, the numbers of active burrows were counted in May and October. If the rodent hole numbers increased, >30% were baited with zinc phosphide. The results showed that changes in the numbers of rodent burrows along the time and incidence rate of ZCL in the intervention and control village were statistically significant[17].

In 2010, rodenticidal effect of Coumavec® (a mixture of Coumatetralyl 0.5% and Etofenprox 0.5%) against *R. opimus* was evaluated under laboratory conditions. The results of this study showed that Coumavec® has some rodenticidal effect on *R. opimus* in laboratory conditions. The authors suggested 0.125% concentration for rodent control operation in the field conditions[18].

A study was conducted from January 2011 to January 2012 to introduce a new alternative rodenticide to control the reservoirs of ZCL in hyperendemic focus of Esfahan. The effect of this operation on the vector density and the incidence of the disease were also studied. Rodent control operation was conducted using zinc phosphide or Coumavec®. Active case findings were done by house-to-house visits once every season. To evaluate the effect of rodent control operation on the vector density, sandflies were collected twice a month using sticky traps. The results showed Coumavec® could be a suitable alternative for zinc phosphide while bait shyness or behavioural resistance is observed[19].

Recently, some behavioural resistance and/or bait shyness against the rodenticide among the great gerbil population has been observed from some endemic foci of the disease (unpublished data, Esfahan Health Centre, Iran). So, it is necessary to introduce some new effective alternative rodenticides to control the reservoir hosts and subsequently the disease in endemic area of ZCL in Iran. The aim of the current study was to introduce an alternative rodenticide to control the reservoirs of ZCL and its effect on the vector density. In this survey, the effect of phostoxin (a fumigant rodenticide) on the main reservoir host and vector of the disease were compared with zinc phosphide 2.5% bait in comparison with the control area.

MATERIAL & METHODS

Study area

The present study was conducted in four villages (Islamabad, Gishi, Vartoon and Parvaneh-Aliabadchi), 50 to 95 km from Esfahan City, Esfahan Province, Iran from April to November 2011. Islamabad (32° 29′ 41.2″ N and 52°16′ 10.9″ E) and Gishi (32° 29’ 19.1″ N and 52° 21′ 13.8″ E) were selected as intervention areas for phostoxin and zinc phosphide, respectively and Vartoon (32° 50′ 07.5″ N and 52° 06′ 51.9″ E) and Parvaneh-Aliabadchi (32° 47′ 46.2″ N and 51° 58′ 27.2″ E) were selected as control areas.

The study areas have an arid climate. In 2010, the maximum mean temperature was 39.1°C and minimum mean temperature was –1.6°C in July and December, respectively. The total rainfall was 72.2 mm. The minimum and maximum mean monthly relative humidity were 7% (July) and 82% (January), respectively.

Rodent control operation

The phostoxin tablet formulation was used in this survey (Prepared by Esfahan Health Centre). The zinc phosphide bait concentration was selected based on the previous studies[16].

In the late April 2011, before the emergence of sandflies, counting and destroying of the rodent burrows were conducted in a radius of 500 m from houses around all study villages. After 48 h the study areas were revisited and the reopened holes were counted again. In treated areas the reopened burrows were baited by phostoxin tablets or zinc phosphide baits and then closed. For phostoxin 1 tablet and for zinc phosphide some 12–15 g of the poisoned baits were put into each burrow in a depth of 10 cm. The study areas were revisited after a week and the reopened burrows in treated areas were counted, baited and closed again. Rodent control operations were carried out monthly in May, June, July and August. The date of baiting and the number of reopened holes were recorded. In Vartoon (control village), no control operation was done, but to compare with intervention areas, the numbers of reopened holes were also counted at each stage.

Entomological surveillance

To evaluate the effect of rodent control operation on the main vector (*P. papatasi)* density, an entomological survey was carried out. Three fixed houses were selected in each village and sandflies were collected by sticky paper traps twice a month from the beginning (April) to the end (October) of sandflies active season. The sticky traps were installed before sunset and collected early the next morning. The collected sandflies were separated from sticky traps, washed with absolute acetone and preserved in 70% ethanol till the time of preparing slides. Microscopic slides of phlebotomines were prepared using Pauri's medium[20] and identified by valid keys[21–22]. Sandflies from outdoor resting places were collected and identified by the same procedure.

Human infection

The effects of rodent control operations on the disease incidence were evaluated. Before and after the intervention, active case findings were carried out in treated (Islamabad and Gishi) and control (Vartoon and Parvaneh-Aliabadchi) villages. As the final goal of the current study was to determine the impact of rodent control operation on the disease incidence, we decided to add an extra control areas (Parvaneh-Aliabadchi) only for active case detection and calculating the incidence of the disease to compare with treated areas. All the selected households (150 households in each treated villages and all the inhabitants, in control villages) were visited in January 2011 and once every season in 2012. Some information such as ID of the people, presence or absence of scar(s) or active lesion(s), number of the lesion(s) or scar(s), and travelling history to the other ZCL foci, were recorded in a questionnaire for each household. Persons who had travelling background to other endemic foci of ZCL were excluded from the survey. New cases of the disease and the number of active lesions were recorded on each visit. At the end of 2011 and 2012, yearly incidences of ZCL in both the treated and control villages were calculated. The persons with scars were excluded from the risk population.

Statistical analysis

STATA and SPSS 16 software were used to analyze the data and graphs were drawn using Excel software. Rodent holes changes and density of sandflies in intervention and control areas were compared using Chi-square test and the Kruskal-Wallis non-parametric tests, respectively.

RESULTS

The treated area of Islamabad (phostoxin intervention area) was around 219 ha and the total number of burrows before intervention was 4985 (23/ha). After 48 h of destroying the rodent burrows, 875 (3.9 active holes/ha) of the holes were reopened. All the reopened holes were baited and closed. After one week of control operations, the number of reopened holes decreased to 262. In June, July and August the reopened burrows were 291, 521 and 589, respectively (Table 1). The treated area of Gishi (zinc phosphide intervention area) was around 193 ha. The number of holes before the intervention was 4729 (24.5/ha). After 48 h of destroying the colonies, the number of reopened holes decreased to 1682 (8.7 active holes/ha). The reopened burrows were baited and closed. After one week, the number of holes reduced to 600. In June, July and August, the number of the burrows was 493, 424 and 704, respectively (Table 1). The control area (Vartoon) was around 173 ha; the number of holes before the intervention was 2297 (13.3/ha) and after 48 h of destroying 196 (1.1 active holes/ha) of these holes were reopened. The reopened holes in this village were not baited or closed. After one week, the number of reopened holes in the control area (Vartoon) increased to 281. In June, July and August the number of burrows increased to 365, 557 and 1306, respectively (Table 1). Compared to the intervention areas, the number of burrows at each stage in control village showed an increasing trend. Reduction rate of rodent holes between the treated villages with phostoxin and zinc phosphide were significantly different (p <0.05).

Total of 4243 adult sandflies (2278 from outdoors and 1965 from indoors) were collected during May to October 2011. Three species were collected from indoors: *P. papatasi* (92.5%), *Sergentomyia sintoni* (7.1%) and *P. sergenti* (0.4%). In outdoor resting places, *P. papatsi* (95.8%), *S. sintoni* (3.3%), *P. sergenti* (0.5%), *P. ansari* (0.2%) and *P. mongolensis* (0.2%) were identified . In all

Table 1. Comparison of the number of rodent holes in the intervention and control villages, Esfahan County, Esfahan, Iran, 2011

Name of village	Treated area (ha)	Pre-treatment	Post- 48 h burrow destruction	One week after first baiting	June	July	August
Islamabad	219	4985	875	262	291	521	589
Gishi	193	4729	1682	600	493	424	704
Vartoon	173	2297	196	281	365	557	1306

the study areas, sandflies emerged in late April and disappeared in late October. The most common and dominant species was *P. papatasi,* in indoor and outdoor resting places. In both the intervention areas with phostoxin and zinc phosphide, monthly density of *P. papatasi* was compared (Figs. 1 and 2). In the village treated with phostoxin, the density of *P. papatasi* in outdoor was lower than that found in indoor resting places. Nevertheless, the density of *P. papatasi* in the village treated with zinc phosphide was higher in outdoors. Statistical analysis showed that there was no significant difference between the density of the vector in indoors and outdoors in intervention and control areas ($p > 0.05$). The yearly incidence of the disease in both treated and control villages is shown in Table 2. The incidence of the disease was calculated at 48.54 and 39.14 per thousand in Islamabad and Gishi (intervention areas) and also 18.40 and 76.19 per thousand in Vartoon and Parvaneh-Aliabadchi (control areas), respectively in 2011. After intervention, the disease incidence dropped to 19.23 and 11.40 per thousand population in Islamabad and Gishi (treated villages), respectively. There was no significant difference between reduction rate of the disease incidence between phostoxin and zinc phosphide ($p > 0.05$). The incidence of ZCL decreased in all the intervention and control villages, as shown in Table 2, but the statistical analysis showed that the reduction rate of ZCL incidence in treated areas was statistically different before and after the intervention ($p < 0.05$). This figure for control area was not significant ($p > 0.05$).

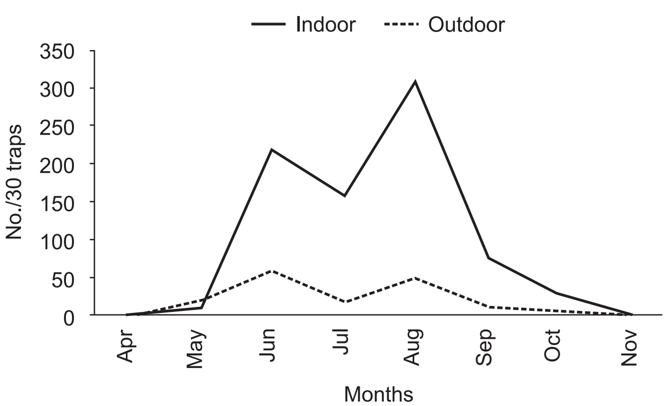

Fig. 1: Monthly fluctuation of *Phlebotomus papatasi* in treated village with phostoxin (Islamabad), Esfahan County, Esfahan Province, Iran, 2011.

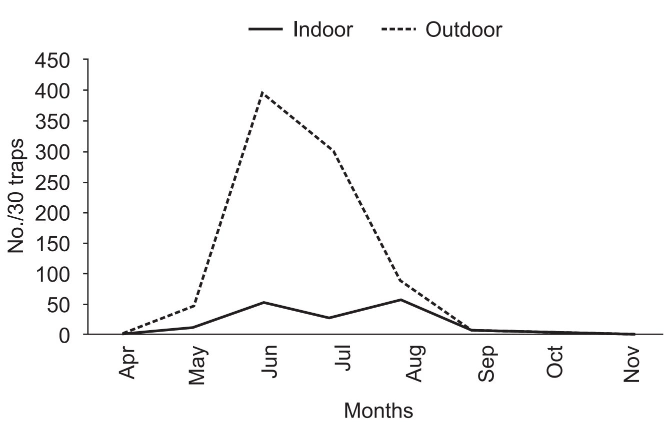

Fig. 2: Monthly fluctuation of *Phlebotomus papatasi* in treated village with zinc phosphide (Gishi), Esfahan County, Esfahan Province, Iran, 2011.

Table 2. Comparison of the incidence (per thousand) of ZCL in the intervention and control villages, Esfahan County, Esfahan, Iran (2011–12)

Name of village	2011		2012	
	No. with AL	Incidence with AL	No.	Incidence
Islamabad (Intervention area with phostoxin)	10	48.54	3	19.23
Gishi (Intervention area with zinc phosphide)	11	39.14	3	11.40
Vartoon (Control area)	3	18.40	2	10.92
Parvaneh-Aliabadchi (Control area)	8	76.19	5	49.5

DISCUSSION

Up to now several measures were employed for ZCL control in Iran such as rodent control operation using rodenticide bait, impregnated bed nets and curtains with pyrethroids, repellents, indoor residual spraying, health education to the community, and leishmanization, during emergency complex situation[11–14]. Till now there is no success in developing an effective vaccine to prevent leishmaniasis[23–26].

The disease incidence shows an increasing trend during the last decade (Zoonosis Department, Ministry of Health and Medical Education of Iran, personal communication). There are several reasons behind this increase such as surveillance system improvement, yearly disease monitoring, people migration from non-endemic areas into the disease foci, presence of different reservoirs, defection in prevention and control operation of the disease and vector control[25]. The results of this study showed that the rodent control operation is an effective measure for decreasing the rodent's population and the disease incidence. Both rodenticides were effective to control the gerbil's population. After intervention, the reduction rate of rodent burrows was 32.68% in the village treated with phostoxin and 58.14% in the village treated with zinc phosphide. It seems that both pesticides are effective on the control of the gerbil population but zinc phosphide is more effective than phostoxin. Nevertheless, by the end of the study, the number of rodent holes in control area (Vartoon) showed 6.66 fold increase. The number of rodent holes trend in the intervention areas decreased while in the control area it increased.

The incidence reduction rates of the disease were 29.31 and 27.74% in Islamabad (treated with phostoxin) and Gishi (treated with zinc phosphide) respectively, therefore both the rodenticides were effective to reduce incidence of the disease. In all studied areas (both intervention and control areas), the incidence of the disease from 2011 to 2012 had a decreasing trend before and after intervention. Results of this study showed that the reduction rates of ZCL in treated areas were significantly different; however, in control areas no significant difference was observed. In the case of requirement of incidence reduction, phostoxin can be an appropriate alternative to zinc phosphide, if necessary.

In a previous study from April to January 1997, a rodent control operation using 2.5% zinc phosphide was conducted to control ZCL. The results showed that reduction rate of ZCL incidence was 12-fold in treated village compared to the control village at the end of the first year and 5-fold at the end of the second year of the operation[16]. From 1999 to 2002, at the same intervention area, another study was conducted to show the effect of rodent control operation on the disease incidence. Results of the study showed that changes in the numbers of rodent burrows in the intervention and control areas were statistically significant. Furthermore, changes in the incidence of ZCL between the intervention and control village were also significant[17].

In Islamabad (treated with phostoxin), the densities of *P. papatasi* in outdoors were lower than indoor resting places. It is reasonable to assume that phostoxin affects the density of *P. papatasi* in outdoors. It appears that this effect is related to the fumigant property of phostoxin. However, the effect of phostoxin on the sandfly, outdoor density had no dramatical effect on incidence reduction rate of the diseases compared to area treated with zinc phosphide. In contrast, in Gishi, the density of *P. papatasi* in indoors was lower than outdoor resting places. The comparison of the density of *P. papatasi* trend in control and treated villages exhibited that rodents control operation has no significant effect on the *P. papatsi* density. Along this survey, in another area Coumavec® (a mixture of Coumatetralyl 0.5% and Etofenprox 0.5%) was evaluated for ZCL reservoir control and the results were compared with zinc phosphide. The reduction rate of rodent holes in intervention areas with Coumavec® and zinc phosphide were 48.46 and 58.15%, respectively. The incidence of ZCL significantly reduced in the treated areas. In area treated with zinc phosphide, the density of *P. papatasi* was higher in outdoors in contrast to that treated with Coumavec®, the density of sandflies was higher in indoors, similar to the results obtained from phostoxin treated area. The results showed phostoxin could be a suitable alternative to zinc phosphide where bait shyness or behavioural resistance has occurred[19].

It is concluded that phostoxin is less effective compared to zinc phosphide, so that this rodenticide can be used only in special circumstances such as lack or ineffectiveness of other rodenticides and only in the colonies far from human and animal dwelling places on small scales. After overcoming bait shyness or behavioural resistance, zinc phosphide should be used again.

ACKNOWLEDGEMENTS

Authors would like to express their thanks to the staff of Esfahan Province Health Centre, Esfahan University of Medical Sciences (EUMS), for their kind collaboration in field operation. We offer our heartfelt thanks to the staff of Esfahan Health Research Centre, National Institute of Health Research, Tehran University of Medical Sciences (TUMS) for their collaboration during the study. This research was financially supported by the School of Public Health, TUMS, Institute for Environmental Research (IER), TUMS, Esfahan Province Health Centre, EUMS and Department of Zoonosis, CDC, Ministry of Health and Medical Education, Islamic Republic of Iran. The authors declare that they have no conflicts of interest.

REFERENCES

1. Akhavan AA. Immune response of great gerbil against *Phlebotomus papatasi* saliva. Saarbrücken, Germany: Lap Lambert Academic Publishing 2011; p. 1–5.
2. Yaghoobi-Ershadi MR, Akhavan AA, Mohebali M. *Meriones libycus* and *Rhombomys opimus* (Rodentia: Gerbillidae) are the main reservoir hosts in a new focus of zoonotic cutaneous leishmaniasis in Iran. *Trans R Soc Trop Med Hyg* 1996; *90:* 503–4.
3. Yaghoobi-Ershadi MR, Javadian E. Epidemiological study of reservoir hosts in an endemic area of zoonotic cutaneous leishmaniasis in Iran. *Bull World Health Organ* 1996; *74*(6): 587–90.
4. Akhavan AA, Mirhendi H, Khamesipour A, Alimohammadian MH, Rassi Y, Bates P, *et al*. Leishmania species: Detection and identification by nested PCR assay from skin samples of rodent reservoirs. *Exp Parasitol* 2010; *126:* 552–6.
5. Akhavan AA, Yaghoobi-Ershadi MR, Khamesipour A, Mirhendi H, Alimohammadian MH, Rassi Y, *et al.* Dynamics of *Leishmania* infection rates in *Rhombomys opimus* (Rodentia: Gerbillinae) population of an endemic focus of zoonotic cutaneous leishmaniasis in Iran. *Bull Soc Pathol Exot* 2010; *103*(2): 84–9.

6. Nadim A, Mesghali A, Amini H. Epidemiology of cutaneous leishmaniasis in the Isfahan province of Iran. *Trans R Soc Trop Med Hyg* 1968; *62*(4): 543–9.
7. Yaghoobi-Ershadi MR, Javadian E. Studies on sandflies in a hyper endemic area of zoonotic cutaneous leishmaniasis in Iran. *Indian J Med Res* 1997; *105:* 61–6.
8. Killick-Kendrick R. Phlebotomine vectors of leishmaniasis: A review. *Med Vet Entomol* 1990; *4:* 1–24.
9. Desjeux P. Information on the epidemiology and control of the leishmaniasis by country or territory. Geneva: World Health Organization 1991 (Unpublished document WHO/LEISH/91.30).
10. Akhavan AA, Yaghoobi-Ershadi MR, Mirhendi H, Alimohammadian MH, Rassi Y, Shareghi N, *et al.* Molecular epizootiology of rodent leishmaniasis in a hyperendemic area of Iran. *Iran J Public Health* 2010; *39*(1): 1–7.
11. Nadim A, Amini H. The effect of antimalaria spraying on the transmission of zoonotic cutaneous leishmaniasis. *Trop Geogr Med* 1970; *22*(4): 479–81.
12. Yaghoobi-Ershadi MR, Moosa-Kazemi SH, Zahraeii-Ramazani, Jalali-Zand AR, Akhavan AA, Arandain MH *et al.* Evaluation of deltamethrin-impregnated bednets and curtains for control of zoonotic cutaneous leishmaniasis in a hyperendemic area of Iran. *Bull Soc Pathol Exot* 2006; *99:* 43–8.
13. Moosa-Kazemi SH, Yaghoobi-Ershadi MR, Akhavan AA, Abdoli H, Zahraei-Ramazani AR, Jafari R*, et al*. Deltamethrin-impregnated bed nets and curtains in an anthroponotic cutaneous leishmaniasis control program in northeastern Iran. *Ann Saudi Med* 2007; *27:* 6–12.
14. Nadim A, Javadian E, Tahvildar-Bidruni GH, Ghorbani M. Effectiveness of leishmanization in the control of cutaneous leishmaniasis. *Bull Soc Pathol Exot* 1983; *76:* 377–83.
15. Momeni AZ, Jalayer T, Emamjomeh M, Khamesipour A, Zicker F, Ghassemi RL, *et al*. A randomised, double-blind, controlled trial of a killed *L. major* vaccine plus BCG against zoonotic cutaneous leishmaniasis in Iran. *Vaccine* 1999; *17*(5): 466–72.
16. Yaghoobi-Ershadi MR, Akhavan AA, Zahraei-Ramazani AR, Javadian E, Motavalli-Emami M. Field trial for the control of zoonotic cutaneous leishmaniasis in Badrood, Iran. *Ann Saudi Med* 2000; *20:* 386–89.
17. Yaghoobi-Ershadi MR, Zahraei-Ramazani AR, Akhavan AA, Jalali-Zand AR, Abdoli H, Nadim A. Rodent control operations against zoonotic cutaneous leishmaniasis in rural Iran. *Ann Saudi Med* 2005; *25:* 309–12.
18. Veysi A, Vatandoost H, Arandian MH, Jafari R, Hosseini M, Yaghoobi-Ershadi MR, *et al*. Laboratory evaluation of a rodenticide-insecticide, Coumavec®, against *Rhombomys opimus*, the main reservoir host of zoonotic cutaneous leishmaniasis in Iran. *J Arthropod-Borne Dis* 2013; *7*(2): 188–93.
19. Veysi A, Vatandoost H, Yaghoobi-Ershadi MR, Arandian MH, Jafari R, Hosseini M, *et al*. Comparative study on the effectiveness of Coumavec® and zinc phosphide in controlling zoonotic cutaneous leishmaniasis in a hyperendemic focus in central Iran. *J Arthropod-Borne Dis* 2012; *6*(1): 18–27.
20. Smart J, Jordan K, Whittick RJ. *Insects of medical importance*. IV edn. Oxford: British Museum, Natural History; Adien Press, 1965; p. 1–295.
21. Theodor O, Mesghali A. On the Phlebotomine of Iran. *J Med Entomol* 1964; *1:* 285–300.
22. Seyedi-Rashti MA, Nadim A. The genus *Phlebotomus* (Diptera: Psychodidae: Phlebotominae) of the countries of the eastern mediterranean region. *Iranian J Public Health* 1992; *21*(1–4): 11–50.
23. Noazin S, Modabber F, Khamesipour A, Smith PG, Moulton LH, Nasseri K, *et al*. First generation leishmaniasis vaccines: A review of field efficacy trials. *Vaccine* 2008; *26:* 6759–67.
24. Noazin S, Khamesipour A, Moulton LH, Tanner M, Nasseri K, Modabber F, *et al*. Efficacy of killed whole-parasite vaccines in the prevention of leishmaniasis: A meta-analysis. *Vaccine* 2009; *27:* 4747–53.
25. Shirzadi M. *Guidelines for control of cutaneous leishmaniasis*. Tehran, Iran: Department of Zoonosis, CDC, Ministry of Health and Medical Education 2010; p. 11–98.
26. Report of the meeting of the WHO expert committee on the control of leishmaniasis. *Control of the leishmaniasis*. *WHO Tech Rep Ser* 949. Geneva: World Health Organization 2010; p. 54–73.

Correspondence to: Mr. Reza Jafari, Esfahan Health Research Station, National Institute of Health Research, Tehran University of Medical Sciences, Esfahan, Iran.
E-mail: jafari_1348@yahoo.com

Received: 15 October 2013 *Accepted in revised form:* 11 July 2014