Research Article

Received: 10 December 2010 Revised: 9 June 2011 Accepted: 26 July 2011 Published online in Wiley Online Library: 20 February 2012

(wileyonlinelibrary.com) DOI 10.1002/ps.2278

Baseline susceptibility of a wild strain of *Phlebotomus papatasi* (Diptera: Psychodidae) to DDT and pyrethroids in an endemic focus of zoonotic cutaneous leishmaniasis in Iran

Zahra Saeidi,[a] Hassan Vatandoost,[a]* Amir Ahmad Akhavan,[a] Mohamad Reza Yaghoobi-Ershadi,[a] Yavar Rassi,[a] Zahra Sheikh,[a] Mohamad Hossein Arandian,[b] Reza Jafari[b] and Ali Reza Sanei Dehkordi[a]

Abstract

BACKGROUND: *Phelebotumus papatsi* is considered to be the main vector of zoonotic cutaneous leishmaniasis as well as sand fly fever in Iran. There are several measures for vector control, with emphasis on insecticides. The objective of this study was to determine the baseline susceptibility of this vector to the commonly used insecticides in an endemic focus of the disease in central Iran. *P. papatasi* collected from the field were used for susceptibility status. Its baseline susceptibility to DDT and pyrethroids was assessed on about 6866 specimens collected from Badrood rural district, Esfahan Province, Iran, during the summer of 2010. The LT_{50} and LT_{90} values were measured according to the World Health Organisation (WHO) test using probit analysis and regression lines.

RESULTS: Results of tests against female *P. papatasi* revealed LT_{50} values to DDT 4%, permethrin 0.75%, deltamethrin 0.1%, cyfluthrin 0.15% and lambdacyhalothrin 0.05% of 1104.97, 182.35, 26.79, 15.42 and 1.48 s respectively. The figures for male *P. papatasi* were 973.51, 59.5, 4.4, 2.65 and 1.5.

CONCLUSION: The results of this study provide a guideline for implementation of different vector control measures. Furthermore, guidelines are needed for monitoring and evaluation of insecticide susceptibility tests against sand flies.

Keywords: *Phlebotomus papatasi*; insecticides; susceptibility; Iran

1 INTRODUCTION

Phlebotomine sand flies (Diptera: Psychodidae) transmit many zoonotic diseases (arboviruses, bartonellosis and especially leishmaniasis) of importance to human health in 88 countries.[1–6] Approximately 1000 species of sand flies are known, and around 70 of these act as leishmaniasis vectors.[7] Worldwide there are an estimated 2 million new cases of leishmaniasis annually, and 12 million people are currently believed to be infected throughout the world [World Health Organisation (WHO)[8]].

There are two types of cutaneous leishmaniasis (CL) in Iran: anthroponotic (ACL) and zoonotic (ZCL). There are some differences between clinical symptoms of ZCL and ACL types of the disease, but sometimes symptoms are not distinguishable. The incubation period for the ACL type is normally longer than for ZCL. The main reservoir hosts of ACL are humans; dogs can act as secondary reservoirs. The main vector is *Phlebotomus sergenti*, and the parasite is *Leishmania tropica*. Zoonotic cutaneous leishmaniasis is distributed in many rural areas of 16 out of 31 provinces of the country. The disease is zoonotic, and rodents of Gerbellinae are the main reservoir hosts. There are different rodents that act as reservoir hosts in various parts of the country, including *Rhombomys opimus, Tatera indica, Meriones hurrianae* and *Meriones libycus*. *Phlebotomus papatasi* is the main vector to humans and rodents; *Leishmania major* is the parasite.[9–27]

Figure 1 shows the number of disease cases in Iran from 1997 to 2009. Owing to improvement of the reporting system by the Ministry of Health and Medical Education of Iran, natural disasters such as earthquakes, urbanisation, the construction of buildings close to colonies of rodents and the immigration of non-immune people to endemic areas, a sharp increase in cases has been reported since 2004. Sandfly fever is another important disease in the country. Infection from the virus has also been detected in

* *Correspondence to: Hassan Vatandoost, Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, PO Box 6446-14155, Tehran, Iran. E-mail: hvatandoost1@yahoo.com; vatando@tums.ac.ir*

a *Department of Medical Entomology and Vetcor Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

b *Isfahan Health Research Station, National Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran*

R. opimus. Human infection from this virus in some parts of the country is high (Javadian E, private communication).

P. papatasi is a widely distributed species throughout the world and is a common species in the Eastern Mediterranean region. ZCL is reported from rural districts in 50% of the 31 provinces in Iran.[28–30] *M. libycus* and *R. opimus* (Rodentia: Gerbillinae) are the main reservoir hosts in the study area, and *P. papatasi* is the vector of *L. major* to humans.[19] *P. papatasi* exhibits a close association with the burrowing rodents that serve as the reservoirs of *L. major*. Rodent burrows are considered to be the primary habitat of immature *P. papatasi* in ZCL foci, and sand fly larvae have been observed feeding on rodent faeces.

The activity of *P. papatasi* in Badrood starts from late April or early May and extends to mid-October with two peaks, one in mid- or late June and the second in early or mid-September.[31]

In the foci of the disease in Esfahan, DDT 75% at a dosage of 2 mg AI m^{-2} as residual spraying was used from 1952 until 1959 for malaria control, but from 1959 onwards, owing to malaria consolidation, the use of DDT was stopped.[16] In foci of ZCL in Iran, control of malaria with DDT yielded no effect on the incidence of leishmaniasis or the sand fly population.[32]

Measures used to control adult sand flies include the use of insecticides for residual spraying of dwellings and animal shelters, space spraying, insecticide-treated nets and curtains and personal protection through application of repellents/insecticides to skin or fabrics. Because the breeding sites of sand flies are generally unknown in Iran, control measures that act specifically against immatures are not feasible, although the effectiveness of a few biological and chemical agents has been demonstrated in laboratory evaluations.[33]

In some circumstances, reservoir control is also recommended. In addition, patient treatment with antiparasite drugs has been prescribed at the acute stage of the disease.[34]

Global warming may cause increased geographic distribution of *P. papatasi*, and the seasonality of the disease and transmission could be extended throughout all months of the year in endemic countries.[35,36]

The objective of this study was to determine the baseline susceptibility of this vector to the commonly used insecticides in an endemic focus of ZCL in central Iran. The results will provide a guideline for implementation of vector control measures.

2 MATERIALS AND METHODS

2.1 Study case

The investigation was carried out during the summer of 2010 in the rural district of Badrood (33° 44′N, 52° 2′E), Natanz County, Esfahan Province, central Iran (Fig. 2). Badrood is situated at an altitude of 1056 m among the foothills of the Karkas Mountains (altitude 3895 m). The area has a desert climate and is very hot in summer and quite cold in winter.

2.2 Sand fly collection

Sand flies were collected by mouth aspirator from 8.00 pm until 2.00 am at different time intervals in Badrood district, Natanz County, Esfahan Province, during the summer of 2010. After collection they were released into the cages and were then transported to the Isfahan Health Research Station for susceptibility tets.

2.3 Susceptibility tests

All the susceptibility tests were carried out according to the WHO recommendation (1981).[37] During the tests, the sand flies were transfered into the exposure tubes at different time intervals, and mortality was then scored after a 24 h recovery period. During the holding time, the insects were supplied with a cotton pad of 20% sucrose solution. All mortalities were corrected according to the results of control with Abbott's correction formula.[38] If the mortality rate in the control group was between 5 and 20%, all mortalities were corrected by Abbott's method. All the tests were ignored when the mortality was higher than 20% in the control group. After each test, all dead and alive sand flies were mounted separately by Pouri's medium for species identification. Males and females were counted separately.

2.4 Paper supplements

The papers impregnated with DDT 4%, deltamethrin 0.1%, permethrin 0.75%, lambdacyhalothrin 0.05% and cyfluthrin 0.15%, as well as the control, were supplied by WHO.

2.5 Statistical analysis

The mortality times of sand flies to insecticides at different time intervals were subjected to Finney's test[39] for calculation of regression lines. From the lines, the LT_{50} and 95% confidence interval as well as the LT_{90} and its 95% confidence interval were calculated. For plotting of regression lines, computer graphics were used.

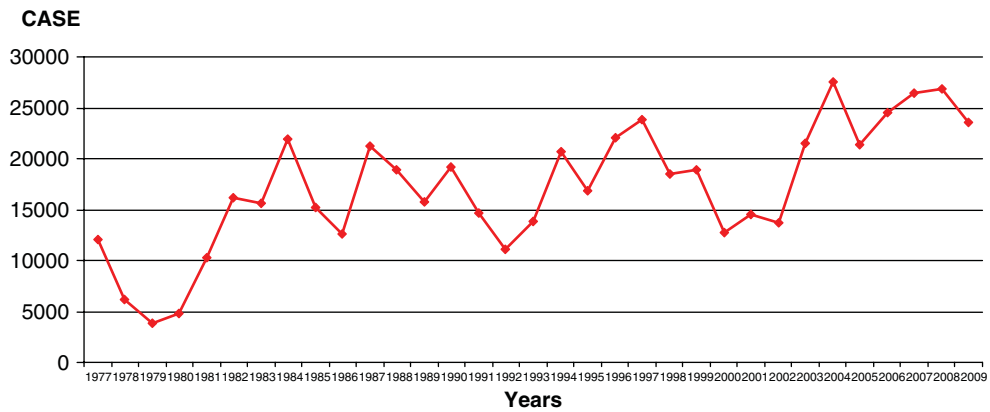

Figure 1. Number of leishmaniasis cases in Iran (Zoonosis Department, Ministry of Health and Medical Education, Iran).

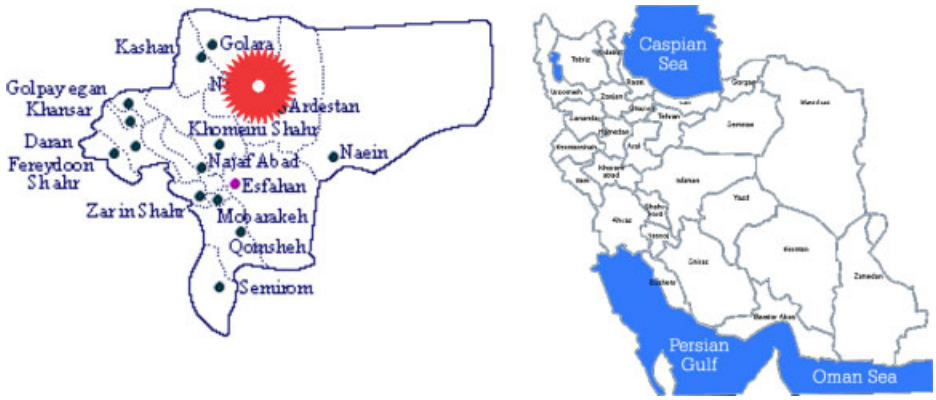

Figure 2. Study area in Badrood rural district, Natanz County, Esfahan Province, Iran.

Table 1. Parameters of probit regression lines of different insecticides against female *P. papatasi*

Insecticide	*A*	*B*± SE	LT_{50}, 95% CI	LT_{90}, 95% CI	χ^2 (df)	Heterogeneity *P*-value	$y = a + bx$
DDT 4%	−7.73	2.54 ± 0.409	711.09	2025	14.35 (3)	<0.05	$y = -7.7296 + 2.5398x$
			1104.97	3531.45			
			1879.25	15 418.4			
Permethrin 0.75%	−5.33	2.36 ± 0.183	159.77	523.25	5.72 (3)	>0.05	$y = -5.3318 + 2.3583x$
			182.35	637.3031			
			207.39	821			
Deltamethrin 0.1%	−1.71	1.20 ± 0.100	19.37	238.7	9.90 (6)	<0.05	$y = -1.7095 + 1.1975x$
			26.79	315.03			
			34.79	447.2			
Cyfluthrin 0.15%	−1.36	1.148 ± 0.126	10.05	147.39	8.92 (4)	<0.05	$y = -1.3627 + 1.1470x$
			15.42	202			
			20.94	315			
Lambdacyhalothrin 0.05%	−0.148	0.866 ± 0.063	0.71	35.62	1.219 (5)	>0.05	$y = -0.1480 + 0.8658x$
			1.48	44.8			
			2.48	58.14			

3 RESULTS

Susceptibility tests against specimens collected in Badrood, Esfahan Province, central Iran, were carried out during the summer of 2010. Table 1 shows the probit regression line parameters for females of *P. papatasi* to different insecticides at the discriminative dose. Figure 3 shows the probit regression lines.

The results of tests against the male vector are presented in Table 2 and Fig. 4.

The results of tests against male *P. papatasi* revealed LT_{50} values to DDT 4%, permethrin 0.75%, deltamethrin 0.1%, cyfluthrin 0.15% and lambdacyhalothrin 0.05% of 973.51, 59.5, 4.4, 2.65 and 1.5 s respectively. The data for females were 1104.97, 182.35, 26.79, 15.42 and 1.48. The results showed that males were more susceptible than females to all the insecticdes tested at the LT_{50} level.

4 DISCUSSION AND CONCLUSIONS

The main measures for ZCL control in Iran are rodent control operation, using impregnated bed nets and curtains with pyrethroids, the use of repellent indoor residual spraying, and health education to the community; during complex emergency situations, leishmanisation is also recommended. According to data released by the Zoonosis Department of the Iranian Ministry of Health and Medical Education, the disease has increased during the last decade. There are several reasons for this increase, which can be summarised as follows: improvement of surveillance systems in the country; monitoring of the disease throughout the year; migration of people from non-endemic regions into disease foci; the occurrence of different reservoirs for ZCL and their migration to non-endemic areas; defects in prevention and in control of the disease and in vector control.

Owing to the lack of information about the susceptibility of sandflies to different WHO-recommended imagicides, the authors conducted a comprehensive field test against the disease vector in the focus of ZCL in Esfahan, Iran. WHO susceptibility tests recommended for mosquitoes were followed. The discriminative doses of DDT and pyrethroids were used in the tests.

In accordance with WHO,[40] the bioassay results for malaria vectors were summarised in three resistance classes: susceptible (mortality 98% or higher); possibly resistant, so called 'tolerant' (mortality between 97 and 80%); resistant (mortality lower than 80%). Results of the present tests against *P. papatasi* at the LT_{50} level revealed that the females need more time than males to be killed at the same concentration. For both sexes, the susceptibility levels to DDT were greater than to pyrethroids. For example, at the LT_{50} level, the required time to gain the same mortality for females against DDT in comparison with lambdacyhalothrin was

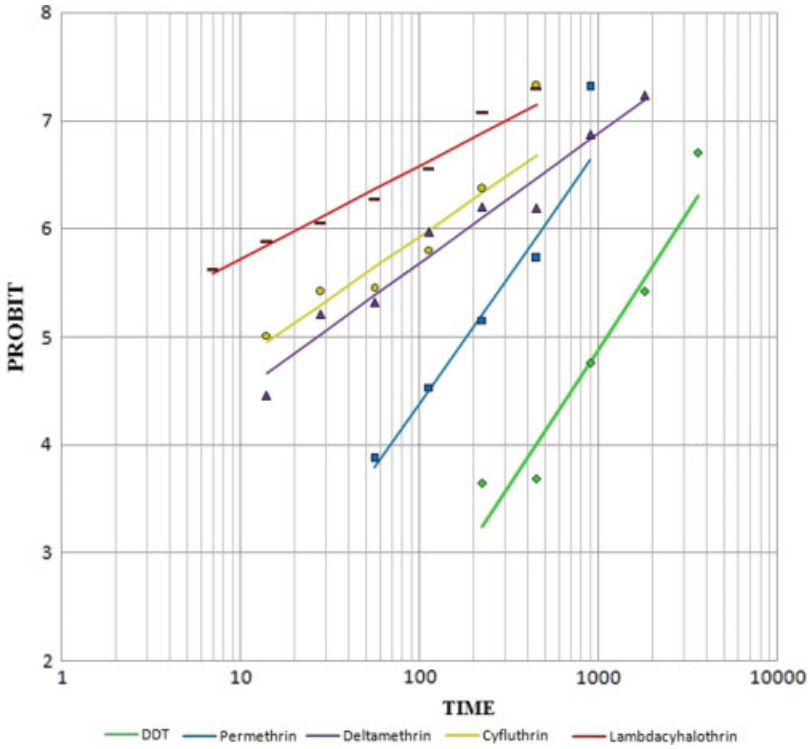

Figure 3. Probit regression lines of different insecticides against female *P. papatasi*.

Table 2. Parameters of probit regression lines of different insecticides against male *P. papatasi*

Insecticide	A[a]	$B\pm$ SE[b]	LT_{50}, 95% CI[c]	LT_{90}, 95% CI[d]	χ^2 (df)	Heterogeneity *P*-value	$y = a + bx$
DDT 4%	−3.99	1.336 ± 0.487	– 973.51	– 8864.77	9.78 (3)	<0.05	$y = -3.9922 + 1.3359x$
Permethrin 0.75%	−2.02	1.136 ± 0.162	– 40.26 59.5 86.29	– 424 799.45 2299.1	4.787 (5)	>0.05	$y = -2.0156 + 1.1359x$
Deltamethrin 0.1%	−0.65	1.016 ± 0.258	0.665 4.4 9.32	42.54 80.29 329.63	0.873 (4)	>0.05	$y = -0.6534 + 1.0159x$
Cyfluthrin 0.15%	−0.33	0.778 ± 0.324	0.0000 2.65 11.07	54.75 117.56 8046.56	0.055 (3)	>0.05	$y = -0.3289 + 0.7779x$
Lambdacyhalothrin 0.05%	−0.174	0.98 ± 0.431	0.0000 1.5 6.03	9.2 30.3 231.37	1 (3)	>0.05	$y = -0.1737 + 0.9824x$

[a] A = intercept.
[b] $B\pm$ SE = slope and its standard error.
[c] LT_{50}, 95% CI = lethal time causing 50% mortality and its 95% confidence interval.
[d] LT_{90}, 95% CI = lethal time causing 90% mortality and its 95% confidence interval.

747 times higher. The figures for permethrin, deltamethrin and cyfluthrin were 6.25-, 41- and 72-fold respectively (see Fig. 5).

The high LT_{50} level of the vector to DDT is attributed to the long-term use of insecticide for malaria vector control in the region. From 1953 for up to 5 years, DDT was applied at a rate of 2 g m^{-2} as indoor residual spraying for malaria control in Badrood. According to the report of the branch of the Ministry of Jihad in Esfahan, several herbicides, fungicides and pesticides have been used for agriculture and veterinary pest control in the region, including malathion, diazinon, fenitrothion, azinphos-methyl, metasystox, carbaryl, permethrin and cypermethrin. There are several reports of susceptibility of *Leishmania* vectors to different insecticides. The authors used WHO criteria as recommended for mosquitoes for the status of susceptibility to sand flies. For instance, the insecticide susceptibility status of *P. papatasi* to DDT, dieldrin, malathion, fenitrothion and propoxur has been estimated in Pali

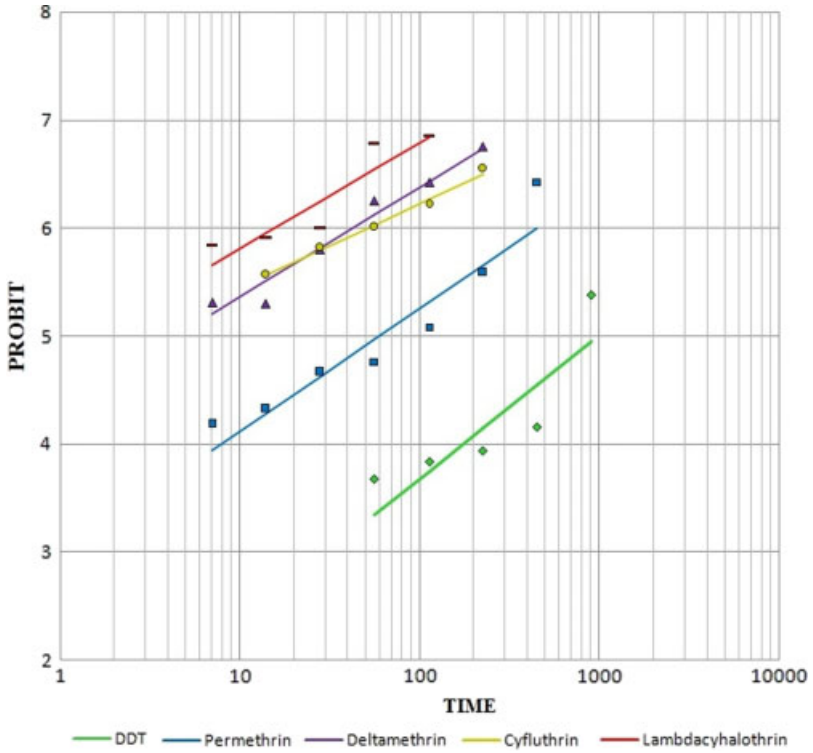

Figure 4. Probit regression lines of different insecticides against male *P. papatasi*.

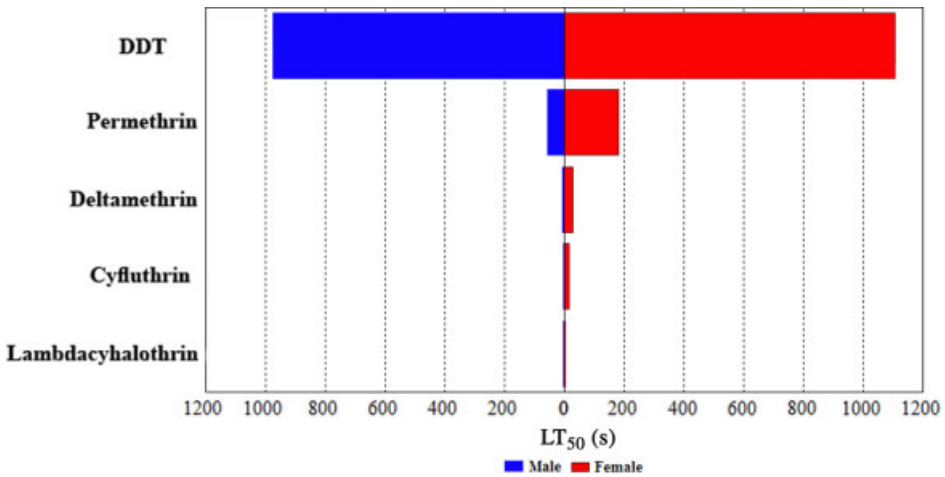

Figure 5. LT_{50} values of different insecticides against male and female *P. papatasi*, 2010.

and Barmer districts of Rajasthan. Tests revealed that this species was resistant to DDT but susceptible to other insecticides.[41] In a similar study in Bikaner district, Rajasthan, the results showed that this species is resistant to DDT, dieldrin and propoxur but susceptible to malathion, fenitrothion and permethrin.[42]

The susceptibility of *P. papatasi* to DDT was studied in field surveys at localities in different areas of Iran during 1985–1988. In many parts of Iran, houses had been treated with DDT for malaria control (1950–1968). Tests were carried out in localities where the application of DDT had been discontinued since 1969. This investigation showed that *P. papatasi* from Esfahan were more tolerant to DDT than flies from other areas, probably a manifestation of DDT resistance.[43] To date, reports of resistance refer to one insecticide (DDT) in only three species (*P. papatasi, P. argentipes* and *Sergentomyia shorti*) in one country (India), although there are reports of increased tolerance to this compound in several countries.[44] There are some reports of DDT resistance in Bihar, India, and Turkey.[45,46]

In the present study, only the susceptibility of *P. papatasi* to different imagicides was compared. Owing to physiological, behavioural and size differences of mosquitoes in comparison with *P. papatasi*, the criteria for resistance status for sand fly is not the same as recommended for the malaria vectors. There is ongoing research into the susceptibility of lab-bred *P. papatasi* to these insecticides in order to find the times of 50% and 100% mortality gain. The authors recommend that the same procedure be used in different parts of the world to pool results and to reach a single conclusion concerning the criteria for susceptibility status

against *P. papatasi*. From the pooled results, the WHO will be able to provide a specific guideline for sand fly, and this guidline will help countries in the monitoring and evaluation of insecticide resistance for implementation of control measures.

ACKNOWLEDGEMENT

The authors would like to express their appreciation to Tehran University of Medical Sciences for kindly providing financial support of this research.

REFERENCES

1 Verani P, Nicoletti L, Ciufolini MG and Balducci M, Viruses transmitted by sand flies in Italy. *Parasitologia* **33**(Suppl.):513–518 (1991).
2 Saidi S, Tesh R, Javadian E, Sahabi Z and Nadim A, Studies on the epidemiology of sand fly fever in Iran. II. The prevalence of human and animal infection with five phlebotomus fever virus serotypes in Esfahan Province. *Am J Trop Med Hyg* **26**:288–293 (1997).
3 Tesh R, Saidi S, Javadian E and Nadim A, Studies on the epidemiology of sand fly fever in Iran. I. Virus isolates obtained from *Phlebotomus*. *Am J Trop Med Hyg* **26**:282–287 (1977).
4 Mehrabi Tavana A, The seroepidemiological studies of sand fly fever in Iran during imposed war. *Iran J Public Health* **30**:145–146 (2001).
5 Sharma U and Singh S, Insect vectors of Leishmania: distribution, physiology and their control. *J Vector Borne Dis* **45**:255–272 (2008).
6 Depaquit J, Grandadam M, Fouque F, Andry PE and Peyrefitte C, Arthropod-borne viruses transmitted by Phlebotomine sand flies in Europe: a review. *Euro Surveill* **15**:1950–1957 (2010).
7 Bates PA, Leishmania sand fly interaction: progress and challenges. *Curr Opin Microbiol* **11**:340–344 (2008).
8 *Leishmaniasis*. [Online]. World Health Organization (2007). http://www.who.int/leishmaniasis [31 January 2012].
9 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* **126**:552–556 (2010).
10 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* **103**:84–89 (2010).
11 Hamarsheh O, Presber W, Yaghoobi-Ershadi MR, Amro A, Al-Jawabreh A, Sawalha S, *et al*, Population structure and geographical subdivision of the *Leishmania major* vector *Phlebotomus papatasi* as revealed by microsatellite variation. *Med Vet Entomol* **23**:69–77 (2009).
12 Yaghoobi-Ershadi MR, Javadian E and Tahvildare-Bidruni GH, The isolation of *Leishmania major* from *Phlebotomus (Paraphlebotomus) caucasicus*, in Esfahan Province, Islamic Republic of Iran. *Trans R Soc Trop Med Hyg* **5**:518–519 (1994).
13 Yaghoobi-Ershadi MR, Javadian E and Kannani A, Host preference pattern of phlebotomine sand flies of Borkhar rural district, Esfahan Province, Iran. *Acta Trop* **60**:155–158 (1995).
14 Yaghoobi-Ershadi MR, Javadian E and Tahvildare-Bidruni GH, *Leishmania major* MON-26 isolated from naturally infected *Phlebotomus papatasi* (Diptera: Psychodidae) in Esfahan Province, Iran. *Acta Trop* **59**:279–282 (1995).
15 Yaghoobi-Ershadi MR and Javadian E, Zoonotic cutaneous leishmaniasis to the north of Esfahan. Human infection in 1991. *Bull Soc Pathol Exot* **88**:42–45 (1995).
16 Yaghoobi-Ershadi MR and Javadian E, Susceptibility status of *Phelebotomus papatsi* to DDT in the most important focus of zoonotic cutaneous leishmaniasis, Esfahan Province, Iran. *Iran J Public Health* **24**:11–19 (1995).
17 Yaghoobi-Ershadi MR and Javadian E, Seasonal variation of *Leishmania major* infection rates in sand flies from rodent burrows in Esfahan Province, Iran. *Med Vet Entomol* **10**:181–184 (1996).
18 Yaghoobi-Ershadi MR and Javadian E, Epidemiological study of reservoir hosts in an endemic area of zoonotic cutaneous leishmaniasis in Iran. *Bull WHO* **74**:587–590 (1996).
19 Yaghoobi-Ershadi MR, Akhavan AA and 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* **90**:503–504 (1996).
20 Yaghoobi-Ershadi MR and Javadian E, Studies on sand flies in a hyperendemic area of zoonotic cutaneous leishmaniasis in Iran. *Indian J Med Res* **105**:61–66 (1997).
21 Yaghoobi-Ershadi MR, Hanafi-Bojd AA, Akhavan AA, Zahrai-Ramazani AR and Mohebali M, Epidemiological study in a new focus of cutaneous leishmaniosis due to *Leishmania major* in Ardestan town, central Iran. *Acta Trop* **79**:115–121 (2001).
22 Yaghoobi-Ershadi MR, Hanafi-Bojd AA, Javadian E, Jafari R, Zahraei-Ramazani AR and Mohebali M, A new focus of cutaneous leishmaniasis caused by *Leishmania tropica*. *Saudi Med J* **23**:291–294 (2002).
23 Yaghoobi-Ershadi MR, Akhavan AA, Zahraei-Ramazani AV, Abai MR, Ebrahimi B, Vafaei-Nezhad R, *et al*, Epidemiological study in a new focus of cutaneous leishmaniasis in the Islamic Republic of Iran. *East Mediterr Health J* **9**:816–826 (2003).
24 Yaghoobi-Ershadi MR, Jafari R and Hanafi-Bojd AA, A new epidemic focus of zoonotic cutaneous leishmaniasis in central Iran. *Ann Saudi Med J* **24**:98–101 (2004).
25 Yaghoobi-Ershadi MR, Akhavan AA, Zahraei-Ramazani AR, Jalali-Zand AR and Piazak N, Bionomics of *Phlebotomus papatasi* (Diptera: Psychodidae) in an endemic focus of zoonotic cutaneous leishmaniasis in central Iran. *J Vector Ecol* **30**:115–118 (2005).
26 Yaghoobi-Ershadi MR, Moosa-Kazemi SH, Zahraei-Ramazani AR, Jalai-Zand AR, Akhavan AA, Arandain MH, *et al*, Evaluation of deltamethrin-impregnated bed nets and curtains for control of zoonotic cutaneous leishmaniasis in a hyperendemic area of Iran. *Bull Soc Pathol Exot* **99**:43–48 (2006).
27 Mohebali M, Javadian E, Yaghoobi-Ershadi MR, Akhavan AA, Hajjaran H and Abaei MR, Characterization of Leishmania infection in rodents from endemic areas of the Islamic Republic of Iran. *East Mediterr Health J* **10**:591–599 (2004).
28 Rassi Y, Javadian E, Jalali M, Motazedian MH and Vatndoost H, Investigation on zoonotic cutaneous leishmaniasis, southern Iran. *Iran J Public Health* **33**:31–35 (2004).
29 Rassi Y, Javadian E, Amin M, Rafizadeh S, Vatandoost H and Motazedian H, *Meriones libycus* is the main reservoir of zoonotic cutaneous leishmaniasis in south Islamic Republic of Iran. *East Mediterr Health J* **12**:474–477 (2006).
30 Rassi Y, Gassemi MM, Javadian E, Rafizadeh S, Motazedian H and Vatandoost H, Vectors and reservoirs of cutaneous leishmaniasis in Marvdasht district, southern Islamic Republic of Iran. *East Mediterr Health J* **3**:686–693 (2007).
31 Yaghoobi-Ershadi MR and Akhavan AA, Entomological survey of sand flies (Diptera: Psychodidae) in a new focus of zoonotic cutaneous leishmaniosis in Iran. *Acta Trop* **73**:321–326 (1999).
32 Seyedi-Rashti MA and Nadim A, Attempts to control zoonotic cutaneous Leishmaniasis in the Esfahan area, Iran. *Iran J Public Health* **2**:199–203 (1974).
33 Alexander B and Maroli M, Control of phlebotomine sand flies. *Med Vet Entomol* **17**:1–18 (2003).
34 Yaghoobi-Ershadi MR, Akhavan AA, Zahraei-Ramazani AR, Javadian E and Motavalli-Emami M, Field trial for the control of zoonotic cutaneous leishmaniosis in Badrood, Iran. *Ann Saudi Med J* **20**:386–389 (2000).
35 Cross ER and Hyams KC, The potential effect of global warming on the geographic and seasonal distribution of *Phlebotomus papatasi* in Southwest Asia. *Environ Health Perspect* **104**:724–727 (1996).
36 Cross ER, Newcomb WW and Tucker CJ, Use of weather data and remote sensing to predict the geographic and seasonal distribution of *Phlebotomus papatasi* in Southwest Asia. *Am J Trop Med Hyg* **54**:530–536 (1996).
37 Instruction for determining the susceptibility or resistance of adult mosquito to organochlorine, organophosphorine and carbamate insecticides – diagnostic test. WHO/VBC/81.806, 1981.1, WHO, Geneva (1981).
38 Abbott WS, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).
39 Finney DJ, *Probit Analysis*, 3rd edition. Cambridge University Press, Cambridge, UK (1971).
40 Report of the WHO informal consultation. Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. WHO/CDS/CPC/MAL/98.12, WHO, Geneva (1998).

41 Singh KV and Bansal SK, Insecticide susceptibility of *Phlebotomus papatasi* to organochlorine, organophosphate and carbamate compounds in some arid areas of western Rajasthan. *Indian J Med Res* **103**:91–93 (1996).
42 Bansal SK and Singh KV, Susceptibility status of *Phlebotomus papatasi* and *Sergentomyia punjabaensis* (Diptera: Psychodidae) to some insecticides in district Bikaner (Rajasthan). *J Commun Dis* **28**:28–32 (1996).
43 Rashti MA, Panah HY, Mohamadi HS and Jedari M, Susceptibility of *Phlebotomus papatasi* (Diptera: Psychodidae) to DDT in some foci of cutaneous leishmaniasis in Iran. *J Am Mosq Control Assoc* **8**:99–100 (1992).
44 Maroli M and Khoury C, Prevention and control of leishmaniasis vectors: current approaches. *Parasitologia* **46**:211–215 (2004).
45 Joshi GC, Kaul SM and Wattal BL, Susceptibility of sand flies to organochlorine insecticides in Bihar (India). Further reports. *J Commun Dis* **11**:209–213 (1979).
46 Resistance of vectors and reservoirs of disease to pesticides. 10th report of the WHO Expert Committee, Vector Biology and Control, Technical Report Series, 737 (1986).

