

## Original Article

# Susceptibility Status of *Phlebotomus papatasi* and *P. sergenti* (Diptera: Psychodidae) to DDT and Deltamethrin in a Focus of Cutaneous Leishmaniasis after Earthquake Strike in Bam, Iran

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## Abstract

**Background:** The cutaneous leishmaniasis (CL) has been occurred in Dehbakri County, located 46 km of Bam District, Kerman Province since 2004–2005. *Phlebotomus papatasi* is an important vector of zoonotic cutaneous leishmaniasis (ZCL) as well as sand fly fever and *P. sergenti* is considered as main vector of anthroponotic cutaneous leishmaniasis (ACL) in Iran. There are several measures for vector control with emphasizing on insecticides. The objective of this study was to determine the baseline susceptibility of leishmaniasis vectors to the DDT and deltamethrin in an endemic focus of CL in southern Iran.

**Methods:** Baseline susceptibility tests were carried out on field collected strains of *P. papatasi* and *P. sergenti* and tested with WHO impregnated papers with DDT 4.0% and deltamethrin 0.05% in the focus of disease in Dehbakri County during summer 2010. The values of LT<sub>50</sub> and LT<sub>90</sub> were determined using probit analysis and regression lines.

**Results:** The LT<sub>50</sub> value of DDT 4.0% and deltamethrin 0.05% against *P. papatasi* was 20.6 and 13.6 minutes respectively. The same data for *P. sergenti* were ranged between 21.8 and 17.7 minutes.

**Conclusion:** The results of tests will provide a guideline for implementation of vector control using pesticides such as impregnated bed nets, indoor residual spraying and fogging.

**Keywords:** *Phlebotomus papatasi*, *Phlebotomus sergenti*, insecticides, susceptibility, Iran

## Introduction

Phlebotomine sand flies are known to transmit varieties of zoonotic diseases including leishmaniasis (protozoa), bartonellosis (bacteria) and sand fly fever (viruses) that affect humans and animals in 88 countries worldwide (Verani et al. 1991, Saidi et al. 1997, Tesh et al. 1997, Mehrabi Tavana 2001, Sharma and Singh 2008, Depaquit et al. 2010). *Phlebotomus papatasi* is widely distributed throughout the world. It is a common species in the Eastern Mediterranean region and considered as important vector of

zoonotic cutaneous leishmaniasis as well as sand fly fever in Iran. Cutaneous Leishmaniasis (CL) is reported from 50% of the 31 provinces in Iran (Yaghoobi-Ershadi et al. 1999, Rassi et al. 2004, Rassi et al. 2006, Rassi et al. 2007, Rassi et al. 2011). The activity of *P. papatasi* and *P. sergenti* in Bam starts from late May and extends to mid October with two peaks, one in mid or late June and the second in early or mid September. *Phlebotomus papatasi* could be a *Leishmania* vector for humans and gerbils and is

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susceptible to DDT (Yaghoobi-Ershadi and Akhavan 1999). Leishmaniasis is endemic in at least 88 poor countries, with the global prevalence of all forms of the disease is 12 million, with 1.5–2 million cases of CL and half a million cases of visceral form.

Current control measures, including environmental sanitation and drug treatment of cases, are expensive and cannot be sustained effectively by poor countries due to the problems of financing and implementation. Moreover, toxicity associated with some of the most widely available drug treatments, including injections of pentavalent antimony compounds, and the resistance developed by the parasite underline the need for development of effective methods of prevention, especially vaccines (Noazin et al. 2008, Noazin et al. 2009). The findings indicate that the whole-parasite vaccine candidates tested do not confer significant protection against human leishmaniasis.

Cutaneous leishmaniasis is still an important public health problem in many parts of the world, where 90% of the cases occur in seven countries including Iran. Two main species of CL are present in Iran; anthroponotic CL (ACL) due to *L. tropica* is the predominant species in many large and medium size cities. The city of Bam has been endemic for many years and a well-known focus for ACL, where recent field trials of autoclaved *L. major* vaccine against ACL were conducted. Zoonotic CL (ZCL) caused by *L. major*, is found in many rural foci in the north, east and south of the country (Sharifi et al. 2010). Emergence or re-emergence of CL has recently occurred in many countries. Both ZCL and ACL have been prevalent in a number of rural and urban areas of Iran. They have also emerged in new foci during recent decades (Fazaeli et al. 2009).

A new emerging focus of ACL due to *L. tropica* in rural areas of Dehbakri County, south-eastern Iran, after the earthquake of 2003 has been confirmed using polymerase

chain reaction (PCR). The current emergence was unexpected in this rural area, where no previous history of ACL was recorded. According to our knowledge this is the first report of a gradually establishing new ACL focus in rural communities after the 2003 earthquake (Sharifi et al. 2011). Nested PCR revealed the first report of occurrence of ACL due to *L. tropica* among 3516 inhabitants in Jiroft City (Pouresmailian et al. 2010).

Molecular and direct detection of *Leishmania* parasites revealed the occurrence of ACL in rural parts of the Bam City after earthquake. Natural disaster completely provides favorable condition for sand fly breeding places as well as propagation of *L. tropica* in the displaced population due to earthquake (Sharifi et al. 2011).

The fauna and monthly activity of the ACL vector has been studied in Bam using the sticky paper traps. The sand fly species identified were *P. sergenti* (77.25%), *P. papatasi* (19.00%), *Sergentomyia baghdadis* (1.69%), *S. sintoni* (1.64 %) and *S. tiberiadis* (0.42%). *Phlebotomus sergenti* was the predominant species, and formed 85.11% and 81.83% of species from the human and animal indoor habitats, respectively. *Phlebotomus sergenti* have two peaks of activity during the year, one of which is from the beginning of July, and the other is at the beginning of September (Aghasi and Sharifi 2003).

According to the Health Center report the main activities for sand fly control was using DDT as Indoor Residual Spraying when there was malaria problem in the region. Subsequently permethrin is used for space spraying. Deltamethrin impregnated bed nets at the dosage of 25 mg/m<sup>2</sup> also had been employed for vector control. The complementary measure was using repellents and covering the lesion with sterile bandage.

Dehbakri County is encountered as the newly emerging ACL foci in Kerman Province since 2003 just after tremendous Bam earthquake. Following this event, some af-

ected people were migrated to Dehbakri Country due to temperate climate compared to Bam City. The prevalence of CL was 213.8 per thousand of people during 2003–2010. In addition, the CL incidence was estimated 34.3 per thousand people at 2010 (unpublished data).

The aim of this study was the determination of baseline data on susceptibility level of *P. papatasi* and *P. sergenti* to DDT and deltamethrin in CL affected villages where the chemical intervention was remained as the main choice for CL control method in the Dehbakri focus, southeast Iran.

## Materials and Methods

### Study area

Dehbakri County is located at the foothill of Jebal-Barez Mountain and 48 km far from southwestern of Bam District (Fig. 1). The population of Dehbakri County was 2470 in 2009. The topography of these localities is foothill with the mean altitude of 2000 m above sea level. This area has a semi-desert climate, temperate at summer and cold at winter. The mean of monthly maximum and minimum temperatures were 40 °C and -5 °C in July and Dec respectively. The total annual rainfall was 220 mm. The minimum and maximum of monthly relative humidity were 45% and 92% respectively in July and Jan.



**Fig. 1.** Dehbakri Country, located in Bam District, Kerman Province, south of Iran

### Sand fly collection

Sand flies were collected using aspirator from 8.00 pm till 2.00 am during summer 2010. The caught sand flies were transferred to the entomological cage and then were kept by wet towel and transported to laboratory research center for implementation of susceptibility tests.

### Susceptibility test

All the susceptibility tests were carried out according to the guideline of WHO (1981). The sand flies were transferred to the exposure tubes and then the mortality was scored at interval logarithmic times and the result was read after 24 hour recovery period. During the holding time, the insects were supplied with cotton pad of water in 10% sugar solution. All tested sand flies were mounted separately using Puri's media in order to identify the species. Male and female sand flies were counted separately.

### Insecticide impregnated papers

Impregnated papers with DDT 4.0% and deltamethrin 0.05%, as well as control papers were supplied by World Health Organization via Iranian Centre for Diseases Management, Ministry of Health and Medical Education of Iran.

### Statistical analysis

The mortality rates were corrected according to the result of control tests using Abbott's formula whereas the control mortality rates were ranged between 5.0–20.0% (Abbott 1925). *Phlebotomus papatasi* and *P. sergenti* were exposed to WHO standard papers of DDT 4.0% and deltamethrin 0.05% at different logarithmic exposure times and the mortalities were recorded after 24 hour and subsequently analyzed by probit analysis (Finney 1971). Goodness of fit of regression lines of *P. papatasi* and *P. sergenti* was measured through the  $\chi^2$  test.  $LT_{50}$  and  $LT_{90}$  values were estimated and the slope values

of the regression lines were calculated. Regression lines were plotted for  $LT_{50}$  values for observed probit of mortalities against exposure times using Microsoft® Excel software ver. 2007.

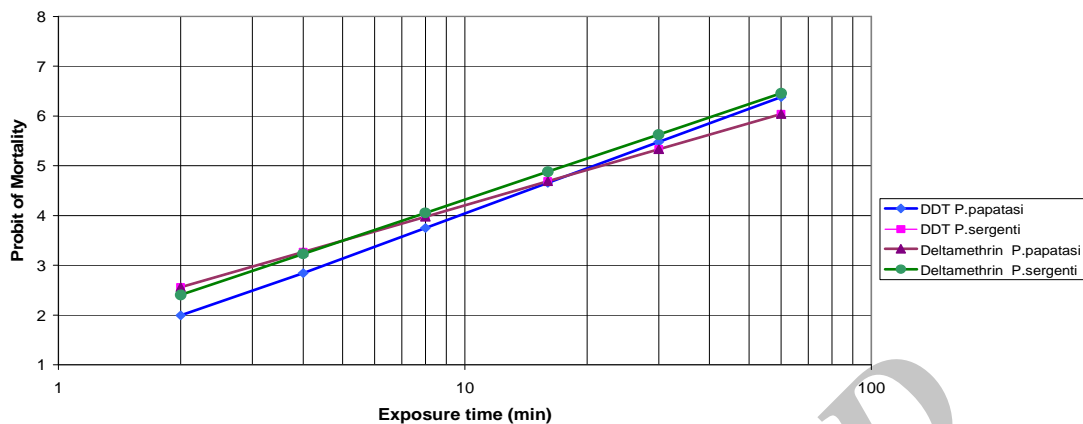
## Results

The mortality rate of field-collected *P. papatasi* with DDT at diagnostic concentration was 100%. The mortality rate for field-collected *P. sergenti* with DDT 4.0% and one hour exposure time was calculated  $97.0 \pm 1\%$ . Considering the criteria of susceptibility for mosquitoes as described by WHO, it should be noted that the tested populations of both *P. papatasi* and *P. sergenti* were also sus-

ceptible to these insecticides. The  $LT_{50}$  values of DDT 4.0% calculated both for *P. papatasi* and *P. sergenti* were 20.7 and 20.8 minutes. The  $LT_{90}$  values of DDT 4.0% were 55.6 and 75.2 minutes respectively for both latter species. The  $LT_{50}$  values of deltamethrin 0.05% calculated both for *P. papatasi* and *P. sergenti* were 13.6 and 17.7 minutes respectively. The  $LT_{90}$  values of deltamethrin 0.05% respectively were 41.9 and 52.0 minutes for *P. papatasi* and *P. sergenti* (Table 1). The regression lines of exposure times of *P. papatasi* and *P. sergenti* which exposed to WHO's impregnated papers with DDT 4.0% and deltamethrin 0.05% are shown in Fig. 2.

**Table 1.** Parameters of regression line of susceptibility of *P. papatasi* and *P. sergenti* of sand flies exposed to two insecticides

Insecticide	Species	No of sand fly tested	Mean of Mortality (%) (diagnostic dose-1 hour)	a (y-intercept)	B (Slope) $\pm$ SE	$LT_{50} \pm 95\%$ C.L.	$LT_{90} \pm 95\%$ C.L.	X2 (Heterogeneity) calculated	X2 (Heterogeneity) table (df)	P. value
DDT4%	<i>P. papatasi</i>	153	100%	-3.9736	3.011 $\pm$ 0.493	16.5127	40.1724	52.724 (4)	18.47 (4)	<0.001
						20.8771	55.6294			
						26.8631	97.1896			
DDT4%	<i>P. sergenti</i>	217	97.0 $\pm$ 1%	-3.1495	2.3547 $\pm$ 0.683	8.5907	32.1209	18.801 (4)	13.28 (4)	<0.001
						21.7519	76.1663			
						115.6334	NS			
Deltamethrin 0.05%	<i>P. papatasi</i>	130	100%	-2.9641	2.6174 $\pm$ 0.414	10.1957	29.8310	5.553 (4)	9.49 (4)	>0.05
						13.5665	41.8909			
						17.7174	73.3799			
Deltamethrin 0.05%	<i>P. sergenti</i>	212	100%	-3.4225	2.7418 $\pm$ 0.352	14.3172	38.4355	7.745(4)	9.49 (4)	>0.05
						17.7120	51.9639			
						22.1612	82.2050			



**Fig. 2.** Probit regression lines of DDT 4% and deltamethrin 0.05% against *P. papatasi* and *P. sergenti* using WHO method at Bam District, southern Iran

## Discussion

Prior of this study, no reports were available on the susceptibility levels of adult *P. papatasi* and *P. sergenti* at the new CL focus of Dehbakri County, Bam District, southern Iran. The susceptibility status of *P. papatasi* to DDT, dieldrin, malathion, fenitrothion and propoxur has been estimated in Pali and Barmer Districts of Rajasthan. The results revealed that this species was resistant to DDT but susceptible to other insecticides (Singh and Bansal 1996). In a similar study in district Bikaner, Rajasthan the results showed that this species was resistant to DDT, dieldrin and propoxur while susceptible to malathion, fenitrothion and permethrin (Bansal and Singh 1996). In Iran, the residual spraying was carried out during 1950–1968 and the houses had been treated with DDT for malaria control. The susceptibility level of *P. papatasi* to DDT was studied at various parts of Iran during 1985–88. Tests were carried out in the localities where the application of DDT had been discontinued since 1969. This investigation showed that *P. papatasi* from Isfahan was more tolerant to DDT than other parts and probably a manifestation of DDT resistance (Rashti et al. 1992). Another study was car-

ried out at 6 villages of Isfahan Province on *P. papatasi* and the values of  $LT_{50}$  and  $LT_{90}$  ranged between 18.10–20.15 minutes and 43.00–63.30 minutes respectively. In the present study, the  $LT_{50}$  values of DDT 4.0% against *P. papatasi* ranged from 20.9 to 55.6 minutes. The same data for DDT 4.0 against *P. sergenti* were ranged between 21.8–76.2 minutes which may related with endophilic habits of fore-mentioned species and exposing the sand flies to chemical used inside the houses. The latter study showed the tolerance in *P. papatasi* population to DDT 4.0% at 1 hour exposure time with mortality rate of 88.8% (Yaghoobi-Ershadi et al. 1995). The susceptibility status of *P. papatasi* had been shown at ZCL foci of Fars Province, southern Iran and the results indicating the highly susceptibility level of this species to DDT in this area (Abai et al. 1993, Rassi et al. 2000). There are several reports revealing DDT resistance at *P. papatasi*, *P. argentipes* and *Sergentomyia shorti* from India (Joshi et al. 1979, Mukhopadhyay, 1992, Maroli and Khoury 2004) as well as *P. papatasi* in Turkey (WHO 1986). In contrast, there are also some reports emphasizing to highly susceptibility levels of *P. papatasi* and *P. ar-*

*gentipes* at different countries (Rahman et al. 1982, Dergacheva et al. 1986, Pener et al. 1987, Kaushal 1995, Fahmy et al. 1996).

Resistance of *P. papatasi* to DDT is first reported in Bihar (Joshi et al. 1979) and then in Turkey (WHO 1986). Mean of LC<sub>90</sub> values for DDT for Egyptian and Sudanese strains of *P. papatasi* ranged from 0.80–1.93% (Schmidt et al. 1969). The LC<sub>50</sub> for DDT for Sudan *P. papatasi* was reported as 0.9% (Qutubuddin 1964). Results obtained from susceptibility tests on field population of *P. papatasi* to DDT revealed that the tested population was highly susceptible to DDT as evidenced by the discriminating concentrations of times. In Iranian foci of zoonotic leishmaniasis, control of malaria with DDT yielded no effect on the incidence of leishmaniasis or the sand fly population (Seyedi-Rashti and Nadim 1974). Measures used to control adult sand fly includes application of insecticides as residual spraying at 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 places of sand flies which are generally unknown, the control measures that act specifically against immature are not feasible, although the effectiveness of a few biological and chemical agents has been demonstrated in laboratory evaluations (Alexander and Maroli 2003). In some circumstances the reservoir control is also recommended. Additionally the patient treatment with anti-parasite drug has been prescribed at the acute stage of disease (Yaghoobi-Ershadi et al. 2000). Tolerance of *P. papatasi* to DDT has been reported from some parts of country (Rashti et al. 1992, Abai et al. 1993, Yaghoobi-Ershadi et al. 1995).

This study showed the higher susceptibility level of *P. papatasi* and *P. sergenti* to deltamethrin 0.05% (LT<sub>50</sub>=13.6–17.7 minutes) and so this insecticides could be effectively used as residual spraying or as ITNs or

LLINs in order to control the CL foci of Iran. Due to epidemic condition of CL, the affected villages of Dehbakri County were sprayed with deltamethrin WP 5.0% at 25 mg ai/m<sup>2</sup> during June 2010.

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