

THE SUSCEPTIBILITY TO 4% DDT AND HOST PREFERENCE OF THE PROBABLE VECTORS OF VISCERAL LEISHMANIASIS IN NORTH WEST OF IRAN

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Abstract

This study was carried out on *Phlebotomus kandelakii* and *Ph.perfiliewi*, the probable vectors of visceral leishmaniasis in the north west of Iran during the summer of 1994.

The results of the susceptibility test showed that the mortality rate with 60-minute exposure to 4% DDT was 100% for each species, while with 30-minute exposure the average mortality rates were 93.3% and 94.4% for *P.kandelakii* and *P.perfiliewi* respectively, indicating the sensitivity of the two probable vectors to DDT in Ardabil Province.

The blood meals of 116 engorged sand-flies were collected from 3 villages of Meshkin-shahr county, including *P.papatasi*, *P.caucasicus* and *P.kandelakii*, and eighty one of them belonged to *P.kandelakii*. All of the blood meals were identified by Enzyme Linked Immunosorbent Assay (ELISA); 33.3% of them were positive with human and 11.1% with dog antisera, indicating a strong preference to man.

Introduction

The first report of the human cases of visceral leishmaniasis (VL) in Iran was from the Caspian area in 1949 (10).

Afterwards, up to the end of 1975 about 120 cases had been reported from different parts of Iran (7). In the last decade more than 1800 cases of the disease had been reported from Ardabil, northwest of Iran (1), and at the present time VL is known as an endemic disease in several foci of at least two Provinces.

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Fars and Ardabil, in the south and the northwest of Iran.

The high number of cases in Bushehr, *Khuzistan* (5), Khorasan (14), East Azarbaijan and Kerman (9) indicate that the disease may also be endemic in these parts of Iran.

The suggested criteria for incrimination of a vector are anthropophily and supporting observations are the demonstration that the sandfly commonly feeds on the reservoir host, which means that the host supports development of the parasite and the fly can transmit the parasite by bite.

On the basis of epidemiological evidence the most suspected vectors of VL in Iran was *Phlebotomus major*, while in recent years *P.kandelakii* and *P.perfiliewi* as well as *P.major* and *P.keshishiani* were found with natural promastigote infection in Ardabil (11) and Fars provinces respectively (12,13).

The purpose of this study was to determine the host preference of the vectors of VL that have not been studied specifically in the foci of the disease in our country and this paper is the first report of anthropophily index of *P.kandelakii* in Iran. Meanwhile, the control of vectors (sand-flies) is the most important methods of the disease control. According to several reports on insect resistance to insecticides, there are no published report on the susceptibility situation of *P.kandelakii* and *P.perfiliewi* and so this study is also the first report from Iran in this regard.

Materials and methods

Sand-flies were collected by aspirators, oiled papers, and C.D.C light traps from human dwellings, stables and dog shelters, in the villages of Ahmad-Abad, Gourt-Tapeh, Gerdeh-Gol (Meshkinshahr district) and Tazeh-Kand, Seyed-Lar (Germi district), in Ardabil Province, north-west of Iran, during 1994.

The head and posterior parts of dissected sand-flies were mounted in a drop of Puri's medium for identification by the method of Theodor and Mesghali (8,14).

The smear of blood meals of each engorged female was prepared on Whatman No.1 filter paper, which was then marked with the number of the sand-fly, place and date of collection. The papers were sent to the protozoology Unit, Department of Medical Parasitology and Mycology in Tehran University of Medical Sciences for ELISA testing (3).

In order to determine the susceptibility level of sand-flies, the tests were performed on a field population of adult females of *P.kandelakii* and *P.perfiliewi* collected from unsprayed indoor resting places of human dwellings, stables and dog shelters. The blood-fed females were caught by active search using a mouth aspirator and torch light. The testing method used was that developed by the World Health Organization (WHO, 1970). DDT impregnated papers were used together with the corresponding control papers.

Ten sand-flies were used for each replicate and for each of the two exposure times (30 and 60 minutes). Sand - flies were held for 24 hours after exposure, and mortalities recorded, and then all dead and living sand-flies were transferred into 70% alcohol for identification.

Results

From the 116 engorged females, eighty one belonged to *P.kandelakii* species. It is suggested that blood-fed females of *Ph.kandelakii* were scarce in this area.

The host preference of this species are presented in Table 1. It is seen that, according to the ELISA test, of the 81 blood meals of *Ph.kandelakii*, the proportion giving a positive reaction from (+) to (++++), with alkaline phosphatase antihuman conjugate, ranged between 15.28 - 100% , with an average of 33.33% , indicating a strong preference for man in this study. The proportions positive for dog, the main animal reservoir host of the disease, were between 6-22% with an average of 11.11%.

The results of the susceptibility of *Ph.kandelakii* and *Ph.perfiliewi transcaucasicus* to 4% DDT are shown in tables 2 and 3.

In the susceptibility tests of 95 females of *Ph.kandelakii* and 98 females of *Ph.perfiliewi transcaucasicus*, the proportions of mortality rates with 60-minute exposure were 100% for each species, while with 30-minute exposure the average mortality rates were 93.3% and 94.4% for those species, respectively (see Table 2 and 3), indicating sensitivity of two species to DDT-impregnated papers. Mean-while, all of the sandflies in the control tubes were alive.

Discussion

As mentioned before, the most important criteria for incrimination of a vector are anthropophily and common infection with the same leishmania parasite

as that found in the same place. In this survey we observed the females of *Ph.kandelakii* feed on humans (33.33%) and domestic dogs (11.11%) and according to finding on natural leptomonaad infection in *Ph.kandelakkii* and *Ph.perfiliewi transcaucasicus*, it is concluded that they are the probable vectors of VL in Ardabil Province, north-west of Iran.

Another finding of this study was the susceptibility level of the two above - mentioned species to 4% DDT solution. We found both, *Ph.kandelakii* and *Ph.perfiliewi transcaucasicus*, sensitive to 4% DDT, and the mortality rates with 60-minute exposure were 100%; the average mortality rates with 30-minute exposure were more than 93% for each species. According to these results, we can use DDT as a suitable insecticide for the control of sand-flies in the human dwellings or the animal shelters.

Table 1- Results of ELISA testing of blood meals of *Ph.kandelakii* (Meshkinshahr-1994)

Name of villages	Date of sand-fly capture	Total number of blood-meals	Human		Dog		Unknown	
			No.P*	%P	No.P	%P	No.	%
Gourt-tapeh	9 July 1994	14	5	35.7	4	28.6	5	35.7
Ahmad-abad	31 July 1994	6	6	100	0	0	0	0
Ahmad-abad	1 August 1994	13	2	15.4	2	9	9	69.2
Ahmad-abad	12 August 1994	22	7	31.8	2	13	13	59.1
Ahmad-abad	24 August 1994	13	5	38.5	0	8	8	61.5
Gerdeh-Gol	27 August 1994	13	2	15.28	1	10	10	76.3
Total no. of blood meals		81	27	33.33	9	11.11	45	55.56

* Positive

Because of the small quantity of blood meals of sand-flies, each of them couldn't be tested against all antisera, so, was tested against human and dog antisera.

Table 3- Results of susceptibility level of *Ph.perfiliewi* to 4% DDT. (Germi, 1994).

Name of village or town	Date of test	Temp. °C min max	Exposure time (minutes)	% Humidity	No of exposed		% Mortality	Control sandflies			
					Total	alive		dead	Total	alive	dead
Germi city	2 July 1994	26 28	60	65	17	0	17	100	11	11	0
"	3 July 1994	26 28	30	65	18	2	16	88.88	10	10	0
Seyed lar	9 July 1994	28 30	60	60	11	0	11	100	9	9	0
Tazeh-Kand	29 June 1994	28 30	30	65	13	0	13	100	9	9	0

Table 2- Results of susceptibility level of *Ph.kandelakii* to 4% DDT. (Meshkinshahr, 1994).

Name of village	Date of test	Temp. °C min max	Exposure time (minutes)	% Humidity	No of exposed		% Mortality	Control sandflies			
					Total	alive		dead	Total	alive	dead
Ahmad- Abad	29 Jun 1994	26 29	60	61	17	0	17	100	12	12	0
	5 Aug 1994	27 29	60	59	30	0	30	100	13	13	0
Ahmad- Abad	10 Aug 1994	25 29	30	58	15	1	14	93.3	9	9	0

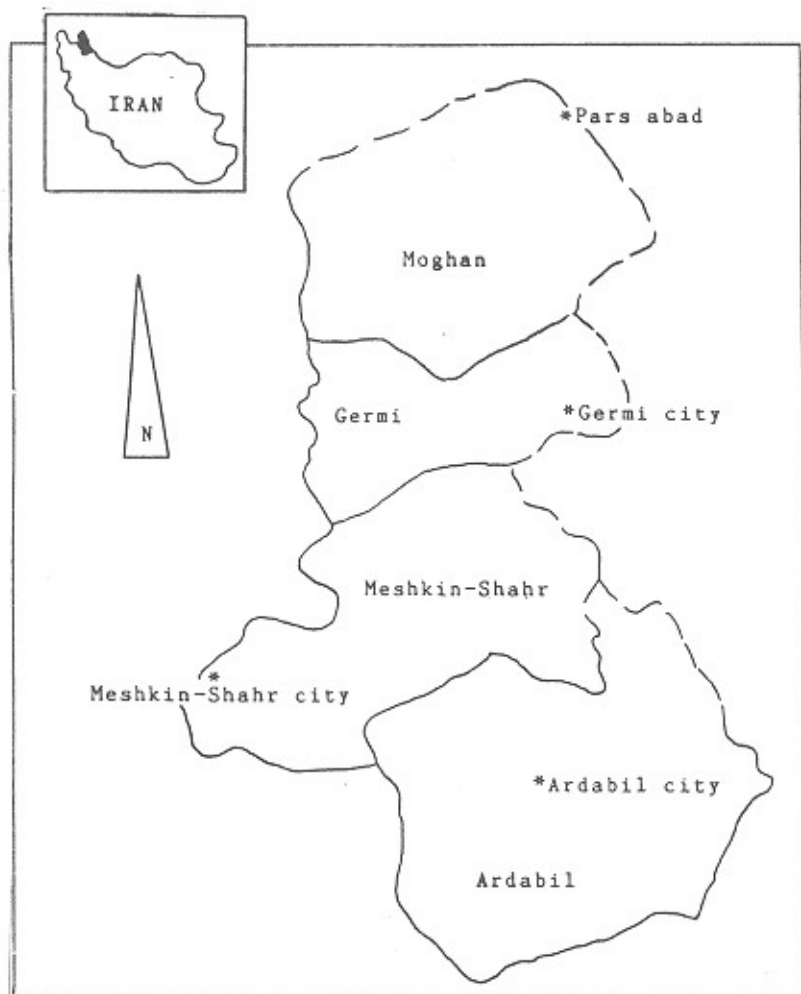


Fig. 1- The map of Ardabil province

References

1. Edrissian, Gh.H., Ahanchin, A.R., Gharachai, A.M., Ghorbani, M., Nadim, A., Ardehali, S., Hafizi, A., Kanani, A., Sarkissian, M., Hajaran, H., Tahvildar-Bidruni, Gh. (1993): Sero epidemiological studies of visceral leishmaniasis and research for animal reservoir in Fars province, Southern Iran, Iranian Journal of Medical Sciences 18,99 - 105.
2. Edrissian, Gh.H., Hafizi, A., Afshar, A., Soleimanzadeh, Gh., Movahed Danesh, A.M. and Garossi, A. (1988): An endemic focus of visceral leishmaniasis in Meshkin - shahr, east Azarbaijan province north west part of Iran and IFA serological survey of disease in this area. Bulletin de la Socie Pathologie Exotique 81, 238 - 248.
3. Edrissian, Gh., Manocheri, A.V. and Hafizi, A. (1985): Application of an Enzyme-linked Immunosorbent Assay (ELISA) for determination of the human blood index in anopheline mosquitoes collected in Iran. J. Av. Mosq. Cont. Assoc. 1, No.3: 349-352.
4. Kilick - kenderick, R. (1990): Phlebotomine vectors of the leishmaniasis: a review. Medical and Veterinary Entomology (1990), 4,1-24.
5. Maraghi, S., Edrissian, Gh.H. and Ebrahimzadeh, A. (1993): Kala-azar in Khusistan. Journal of Medical Faculty, Guilan University of Medical Sciences 1, 1-7 [in Persian].
6. Nadim, A., Javadian, E., Tahvildar - Bidruni, Gh., Mottagi, M. and Abai, A. (1992): Epidemiological aspects of Kala-azar in Meshkin-shahr, Iran, investigation on vector, Iranian, J. Publ. Hlth 21, 61-68.
7. Nadim, A., Navid-Hamidi, A., Javadian, E., Tahvildar-Bidruni, Gh. and Amoo, H. (1978): Present status of Kala-azar in Iran. American Journal of Tropical Medicine and Hygiene 27, 25 - 28.
8. Nadim, A. and Javadian, E. (1976): Key for species identification of sand-flies (*Phlebotominae*, *Diptera*) of Iran. Iranian. J. Publ. Hlth. 5, 33 - 44.
9. Niknaphs, P., Daei parizi, M. Hand Ahmadi, A. (1993): Report of 40 cases of Kala-azar from Kerman province. Journal of Kerman Univeristy of Medical Sciences 1,30-37 [in persian].
10. Pouya, Y. (1949 a): Kala - azar in Iran. Journal of Faculty of Medicine of Tehran, 7, 115-121 [in Persian].
11. Rassi, Y., Javadian, E., Nadim, A., Tahvildar - Bidruni, Gh. Natural leptomonad infection of sand-flies with its first occurrence in *S.dentata* in

- Ardabil province, north west of Iran, Iranian. J. Publ. Hlth (in press).
12. Sahabi, Z., Seyedi-Rashti, M.A., Nadim, A., Javadian, E. and Kazemini, M.R. (1992): Preliminary report on the natural leptomnad infection of *Ph.major* in an endemic focus of visceral leishmaniasis in Fars provinces, south of Iran. Publ, Hlth, 21, 87-93.
 13. Seyedi Rashti, M.A., Sahabi, Z., Kanani, A. *Phlebotomus keshishiani*, a new vector of visceral leishmaniasis in Iran. Iranian. J. Publ. Hlth (in press).
 14. Tabatabestani, M., Elahi, R. and Fata, A.M. (1991): Retrospective and Prospective Studies of Kala-azar in 40 patients in Mashhad University Hospitals. Medical Journal of Mashhad 37, 5-20 [in Persian].
 15. Theodor, O. and Mesghali, A. (1964): On the Phlebotomine of Iran. J. Med. Ent., 1, No. 3, 285 - 300.