Original Article

Diversity of Sand Flies (Diptera: Psychodidae) in Endemic Focus of Visceral Leishmaniasis in Azar Shahr District, East Azarbaijan Province, North West of Iran

Teimour Hazratian [1], Hasan Vatandoost [2], Mohammad Ali Oshaghi [2], Mohammad Reza Yaghoobi-Ershadi [2], Esmael Fallah [1], Sayena Rafizadeh [3], Mohammad Reza Shirzadi [3], Mansoreh Shayeghi [2], Kameran Akbarzadeh [2], *Yavar Rassi [2]

[1]Departmemt of Parasitology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[3]Ministry of Health and Medical Education, Tehran, Iran

(Received 3 Aug 2014; accepted 17 Aug 2014)

Abstract

Background: There are nearly 1000 species of Phlebotomine sand flies in 6 genera, of which only two, *Phlebotomus* in the old world and *Lutzomyia* in the new world are medically important. Globally, leishmaniasis prevalent in 98 countries and affects estimated 12 million people with almost two million new cases per year. Some rural areas of Azarshahr District in East Azarbaijan Province have been reported to be endemic for visceral leishmaniasis. This study is the first attempt to determine the species diversity and density in a new focus of visceral leishmaniasis in Azarshahr District, East Azarbaijan Province, Iran.

Methods: Sand flies were collected form indoor and outdoor biweekly using sticky traps. Diversity index of the collected sand flies within different villages were estimated by the Shannon-Weaver.

Results: The activity of the sand flies extended from April to October with one peak in August. Diversity of sand flies within study area were estimated as 0.917, 1.867, 1.339, 1.673, and 1.562 in Almalodash, Jaragil, Segaiesh, Amirdizaj, and Germezgol Vvillages, respectively.

Conclusion: Identifying the diversity and seasonal abundance of the collected species is of importance for prediction of the period of maximum risk for leishmaniasis transmission and for the successful implementation of a control program. Species diversity is one of the most important factors in ecological studies·

Keywords: Diversity, Density, Sand flies, Visceral Leishmaniasis, Azar Shahr, Iran

Introduction

Phlebotomine sand flies belong to the family Psychodidae (Young and Duncan 1994). Psychodidae family is characterized by numerous parallel veins running to wing margin and the presence of dense hairs on the wings and thorax. From 5 Psychodidae subfamilies, Phlebotominae is known to Phlebotomine sand flies by biting mouth parts that are longer than the head, five-segmented palps, nearly cylindrical antennae, a five-brached radial vein on the wing, and the absence of an eye-bridge (Lane and Crosskey 1993). Generally, sand flies are distinguished from other small flies by their size (2.5–3.5mm in length), characteristic hopping flight, and the "V" position in which they hold their wings while resting (Triplehom and Jhonson 2005).

In the old world, about 40 species of *Phlebotomus* have been proven or suspected vectors of *Leishmania* (Rassi 2004). In Iran, 44 species of the sand flies are found and the

*Corresponding author: Prof Yavar Rassi, E-mail: rassiy@tums.ac.ir

http://jad.tums.ac.ir
Published Online: January 06, 2016

presence of other 10 species is doubtful. In the endemic center of northwest of Iran, two species of *Ph. kandelakii* and *Ph. Perfiliewi* and in the south of Iran, three species of, *Ph. keshishiani*, *Ph. alexandri* and *Ph. major* are the vectors of the disease (Rassi 2007, Azizi 2009).

Leishmaniasis is prevalent in 98 count-ries. This parasitic disease is known one of ten major diseases by WHO (Maia et al. 2011). It has widely spread not only in tropical countries but also in some regions with very different topography (Faiman 2000, WHO 2010). Clinically, leishmaniasis is observed in four types: Visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), mucocutaneus leishmaniasis (ML) and difuse cutaneous leishmaniasis (DCL) (WHO 1994). From 4 mentioned types, only two types of visceral and cutaneous leishmaniasis are common in Iran. Visceral leishmaniasis is considered as the main problems in Iran (Rassi 2011). This disease is observed in all areas of Iran sporadically but the endemic foci of the disease have been reported from Ardabil, Fars, Boshehr and East Azerbaijan Provinces (Rassi et al. 2005, Oshaghi et al. 2009, Rassi et al. 2010).

Some rural areas of Azarshar District in East Azarbaijan Province have been reported to be endemic for visceral leishmaniasis with more than 34 new cases. Performing the control plans without sufficient information about the fauna and ecological parameters is not possible. Therefore, this study was carried out during late April to late October 2010 in rural areas of Azarshahr District, East Azarbaijan Province, Northwestern Iran, to identify diversity, species composition and other ecological parameters.

Material and Methods

Study area

Azar Shahr is located 45 km far from Tabriz. The geographical coordinates is 45 30′E and 37˚30′N. The height of this county from the sea level is 1368 meters. It is limited to Lake of Urmia from the west, to Ajabshir City from the south, to Osko from the north and to Sahand Mountains from the east. In this county, the average rainfall and the relative humidity were 303 and 49 millimeters, respectively. The mean of the annual temperature is 15.25 and the mean of monthly maxi-mum and minimum temperatures were 27.7 and 3.7 ℃ respectively. The population of this county has been 103952 people in 2006.

Selection of Study villages

According to reported cases of the disease, a number of villages including: Segaiesh, Amirdizaj, Almalodash, Jaragil and Germezgol were selected from the county of Azarshahr in east Azarbaijan Province.

Segaiesh village: It is located 14 km from Azar Shahr with 183 families and 633 people in 2010.

Amirdizaj Village: it is located 16 km from Azar Shahr with 250 families and 1046 people.

Almalodash Village: It is located 20 km from Azar Shahr with 182 family and 860 people.

Jaragil Village: It is located 15 km from Azar Shahr with 110 families and 422 people.

Germezgol Village: It is 12 km from Azar Shahr with 70 families and 300 people in 2010.

Sand fly sampling

Sand flies were collected using sticky traps (castor oil coated white papers 20 cm× 30 cm) biweekly from selected villages indoors (bedrooms, guest room, toilet and stable) and outdoors (rodent burrow). Traps (60 papers per village) were installed in sunset and sand flies were collected during sunrise. Sample collection began in late

http://jad.tums.ac.ir
Published Online: January 06, 2016

April and continued until late October 2010, when sand fly activities were reduced sharply. Collected sand flies were stored in 96% ethanol. In the laboratory for species identification, head and three last abdomen segments of females, head with whole body of males specimens were mounted in Puri's medium. Species identification were carried out after one week using keys of sand flies (Rassi and Hanafi bojd 2006).

Determination of Density

The density of sand flies collected by sticky traps was calculated using the formula number of specimens / M^2 of sticky traps and numbers of specimens/ number of traps. Logistic regression used to investigate the existence of a correlation between the abundance of the sand flies, and average monthly temperature in the study area using SAS software (Kasap et al. 2009, Tarallo 2009).

The density of species was corrected according to the formula (Guernaoui 2009, Rajider 2010)

$$\text{Density} = \sqrt{1 + \frac{number\ of\ specimens}{number\ of\ traps}}$$

Determination of the Species Diversity

To calculate the species diversity in a population, there are different methods but in most resources, Shannon-Weaver index has been used. Shannon-Weaver index is shown as $H' = -\sum_{i=1}^{S} = Pi\ Loge\ pi$. In this formula, S is the Richness of the species and pi is the proportion of species Ith in the population and log e is common logarithm. To convert it to common logarithm, it should be multiply in 2.303. In the present study, it is used Shannon-Weaver formula (H').

Species diversity composed of two factors, species richness and species evenness. There are many kind of species diversity indexes, a common one is the Shannon-Weaver index:

$(H' = \sum_{i=1}^{s}$ Pilogepi)

H′= The diversity index
Pi= The proportion of the Ith species
Loge= Natural log
S= Number of species in the community

Results

According to formula, monthly average density, number of specimens/ m2 of sticky traps were 37.60, 41.10, 40.23, 30.38 and 30.67 in Almalodash, Jaragil, Segaieesh, Amirdizaj and Germezgol, respectively. Corrected monthly average density, number of specimens/ number of traps, in mentioned villages were 1.47, 1.51, 1.39 and 1.40.

Table 1. Density of sand flies in studied villages in Azarshahr District, Eastern Azarbaijan, Iran, 2010

Village	Mean density= $\frac{Number\ of\ Specimens}{Number\ of\ traps}$	Mean density= $\frac{Number\ of\ Specimens}{M^2\ of\ traps}$	Mean density= $\sqrt{1+\frac{Number\ of\ Specimens}{Number\ of\ traps}}$
Almalodash	1.18	37.60	1.47
Amirdizaj	0.95	30.38	1.39
Jaragil	1.29	41.10	1.51
Segaiesh	1.26	40.23	1.51
Germezgol	0.96	30.67	1.4

To calculate the species diversity, we used the formula Shannon-Weaver because it has been used in most resources to calculate diversity. The results are shown in tables 2, 3, 4, 5 and 6.

http://jad.tums.ac.ir
Published Online: January 06, 2016

Table 2. Diversity of sand flies in Almalodash Village in Azarshahr District, Eastern Azarbaijan, Iran, 2010

Community A (Almalodash)				
Species	**Number**	**Proportion (Pi)**	**Log$_e$ pi**	**Pilog$_e$ pi**
Ph. papatasi	554	0.54	-.666	-.333
Ph.sergenti	77	0.08	-2.526	-.202
Ph.kandelakii	58	0.06	-2.514	-.169
***Ph.(Adlerius)* spp**	9	0.01	-4.606	-.046
Ph.C.group	203	0.19	-1.661	-.316
Ph.halepensis	78	0.07	-2.650	-.186
Ph.longiductus	19	0.02	-3.913	-.078
Ph.major	19	0.02	-3.913	-.078
Ph.mongolensis	9	0.01	-4.606	-.046
Total	1026	$H' = -\sum_{i=1}^{S} = Pi\ Loge\ pi = 1/454$		

Table 3. Diversity of sand flies in Amirdizaj Village in Azarshahr District, Eastern Azarbaijan, Iran , 2010

Community B (Amirdizaj)				
Species	**Number**	**Proportion (Pi)**	**Log$_e$ pi**	**Pilog$_e$ pi**
Ph. papatasi	214	0.211	-1.556	-.328
Ph.sergenti	232	0.229	-1.474	-.337
Ph.mesghali	9	0.009	-1.744	-.442
Ph.c.group	175	0.173	-1.754	-.303
Ph.mongolensis	145	0.143	-1.945	-.278
Ph.tobbi	145	0.143	-1.945	-.278
Ph.kandelakii	9	0.009	-0.411	-.042
Ph.halepensis	19	0.018	-4.081	-.072
Ph.andrejevi	39	0.038	-3.271	-.124
Ph.caucasicus	19	0.018	-4.018	-.072
Ph.pawlowskyi	9	0.009	-4.711	-.042
Total	1015	$H' = -\sum_{i=1}^{S} = Pi\ Loge\ pi = 1/918$		

Table 4. Diversity of sand flies in Jaragil Village in Azarshahr District, Eastern Azarbaijan, Iran, 2010

Community C (Jaragil)				
Species	**Number**	**Proportion (Pi)**	**Log$_e$ pi**	**Pilog$_e$ pi**
Ph. papatasi	67	0.005	-2.901	-.159
Ph.sergenti	378	0.313	-1.126	-.363
Ph.tobbi	28	0.023	-3.733	-.087
Ph.kandelakii	19	0.016	-4.136	-.006
Ph.major	9	0.007	-4.963	-.035
Ph.c.group	369	0.305	-1.187	-.362
Ph.hodsoni	39	0.032	-3.343	-.110
Ph.mongolensis	184	0.152	-1.884	-.286
Ph.halepensis	97	0.080	-2.252	-.202
Ph.longiductus	9	0.007	-4.963	-.035
Ph.andrejevi	9	0.007	-4.963	-.035
Total	1208	$H' = -\sum_{i=1}^{S} = Pi\ Loge\ pi = 1/74$		

http://jad.tums.ac.ir
Published Online: January 06, 2016

Table 5. Diversity of sand flies in Segaiesh Village in Azarshahr District, Eastern Azarbaijan, Iran, 2010

Community D (Segaiesh)				
Species	**Number**	**Proportion (Pi)**	**Log_e pi**	**$Pilog_e$ pi**
Ph. papatasi	438	0.540	-0.616	-.333
Ph.tobbi	9	0.011	-4.514	-.049
Ph.sergenti	38	0.047	-3.058	-.144
Ph.c.group	194	0.239	-1.432	-.342
Ph.hodsoni	9	0.011	-4.511	-.049
Ph.kandelakii	38	0.047	-3.05	-.144
Ph.mongolensis	48	0.059	-2.831	-.167
Ph.halepensis	19	0.023	-3.733	-.167
Ph.perfiliewi	9	0.011	-4.511	-.049
Ph.major	9	0.011	-4.511	-.049
Total	811	$H' = -\sum_{i=1}^{S} = Pi\ Loge\ pi = 1/413$		

Table 6. Diversity of sand flies in Germezgol Village in Azarshahr District, Eastern Azarbaijan, Iran, 2010

Community E (Germezigol)				
Species	**Number**	**Proportion (Pi)**	**Log_e pi**	**$Pilog_e$ pi**
Ph. sergenti	156	0.269	-1.313	-.353
Ph.ansari	9	0 .016	-4.136	-.066
Ph.kandelakii	9	0·016	-4.136	-.066
Ph.c.group	9	0·016	-4.136	-.066
Ph.halepensis	48	0·083	-2.248	-.207
Ph.mongolensis	223	0.385	-.955	-.368
Ph.tobbi	19	0.033	-3.412	-.113
Ph.andrejevi	68	0.117	-2.146	-.251
Ph.caucasicus	29	0.050	-2.996	-.149
Ph.perfiliewi	9	0·016	-4.136	-.066
Total	811	$H' = -\sum_{i=1}^{S} = Pi\ Loge\ pi = 1/413$		

Due to the tables of species diversity, it is obvious that the species diversity in Amirdizaj Village is in highest level than other villages and the diversity of the sand flies in this village (Population B) has more diversity and better results.

Discussion

This study is the report of the results of entomology investigations on sand flies in Azarshahr District. The geographical location of this district is appropriate for the population being potentially at risk for visceral leishmaniasis.

According to Table 1, the corrected density of sand flies in Jaragil and Segaiesh villages had highest level (1.51) and we found the lowest level (1.39) in Amirdizaj village. The monthly activities of sand flies started from first half of June and have been continued to second half of October.

The most level of density was related to August. Whereas, there was a peak of temperature in this month. In another words,

http://jad.tums.ac.ir
Published Online: January 06, 2016

the population of the sand flies dynamically was direct correlation to the temperature of the area but to the humidity. Given the history of the emergence of the sand flies adults in the region (20 May, 2010) and the density peak of in this region, it can be determined the control programs. Especially, if there is chemical control program, we can determine exact date of residual spraying reasonably and avoid unreasonable residual spraying, further costs and extreme residual spraying.

Among 16 species collected and identified, *Ph. ansarii* and *Ph. mesghali* had lowest density (0.18%). According to the studies of Rassi et al. 2000 on the fauna of the sand flies in west north of Iran and Kaverizadeh et al. 2000 on the fauna of sand flies in Ahar, all 16 species have been reported first time in Azarshahr. Furthermore, two species of *S. hodgsoni* and *P. mesghali* have been observed in East Azerbaijan.

Two main factors of species diversity are: Richness of species and Evenness of species. Richness of species is the species diversity in the population and the Evenness of species is the species diversity of the people in the species.

The results are shown in tables 2, 3, 4, 5 and 6. In Al-Baha Province in the Saudi Arabia, a similar study has been performed (DohaandSomy 2010) with bionomics relation of Phlebotomine sand flies population with temperature and species diversity in different places that its result is compatible to our results of this study.

Conclusion

According to the results, most species diversity was observed in Amirdizaj, therefore, this village can be the best choice for determine of species composition of sand flies in the study area. It should be mentioned, identifying the diversity and seasonal abundance of the collected species is very important for prediction of the period of maximum risk for leishmaniasis transmission and for the successful implementation of a control program.

Acknowledgements

The authors are grateful to Mohammad Reza Abai and Ahmad Ali Hanafi Bojd (Academic members of Tehran University of Medical Sciences) and staffs of Azarshahr Center of Disease Control, Eastern Azarbaijan Province, Iran for their contribution and help in sand fly collection and identification. This work was financially supported by Tehran University of Medical Sciences, Project No.10515, 2010

References

Azizi K, Rassi Y, Javadian E (2006) *Phlebotomus* (*paraphlebotomus*) *alexandri* a probable vector of *Leishmania infantum* in Iran. J Trop Med Parasitol. 100(1): 63–68.

Doha SA, Somy AM (2010) Bionomics of phlebotomine sand fies (Diptera: Psychodidae) in the province of Al-Baha, Soudi Arabia. Med Inst, Oswaldo Cruz, Riodejanerio. 105(7): 850– 856.

Faiman R, Cuno R, Warburg A (2009) Control of phlebotomine sand flies with Vertical fine-Mesh Nets. J Med Entomol. 46(4): 820–831.

Guernaoui S, Boumezzough A (2009) Habitat preferences of phlebotomine sand flies in southwestern Morocco. J Med Entomol. 46(5): 1187–1194.

Hazratian T, Rassi Y, Oshaghi MA, Yaghoobi-Ershadi MR, Fallah E, Shirzadi MR, Rafizadeh S (2011) Phenology and population dynamics of sand flies in a new focus of visceral

http://jad.tums.ac.ir
Published Online: January 06, 2016

leishmaniasis in Eastern Azarbaijan Province, north western of Iran. Asian Pac Trop Med. 4(8): 604–609.

Kasap OE (2009) Activity paterns of sand fly (Diptera: Psychodidae) species and comparative Performance of different trap in an endemic cutaneous leishmaniasis focus in Cukurrova plain, southern Anatolia, Turkey. Acta Vet Br. 78: 327–335.

Maia C, Seblova V, Sadlova J, Votypka J, Volf P (2011) Experimental transmission of *Leishmania infantum* by two magor vectors: a comparison between, viscerotropic and a dermotropic strain. Plos Negl Trop Dis. 5(6): e1181.

Oshaghi MA, MR Yaghobi-Ershadi, M Abbassi, P Parvizi, AA Akhavan, A Rahimi Foroshani, AR Zahraei, Y Rassi, F Mohtarami (2008) Detection of Leishmania major In Naturally Infected Sand Flies Using Semi Nested-PCR. Iran J Publ Health. 37(4): 59–64.

Oshaghi MA, Maleki Ravasan N, Javadian E, Rassi Y, Sedraei J, Enayati AA (2009) Vector incrimination of sand files in the most important vis-ceral leishmaniasis focus in Iran. Am J Trop Med Hyg. 81(4): 527–577.

Papa A, Konstantinou G, Pavlidou V, Antoniadis A (2006) Sand fly fever virus outbreak in Cyprus. Clin Microbiol Infect. 12: 192–194.

Rajinder S, Phlip E (2010) The seasonal abundance of phlebotomine sand flies, *Lutzomyia* species in Florida. J Am Mosq Control Assoc. 26(1): 10–170.

Rassi Y (2011) First detection of *Leishmania infantum* in *Phlebotomus kandelakii* using molecular methods in North Eastern Iran. 7th international sympo-sium on Phlebotomine sand flies, Tur-key. 25–30 April, 2011.

Rassi Y (2007) The Seminested PCR based detection of *Leishmania infantum* infection in asymptomatic dogs in a new endemic focus of visceral leishmaniasisn in Iran. Iran J Arthropod-Borne Dis. 1(1): 38–42.

Rassi Y, Firouzi R, Javadian E (2009) Position of visceral leishmaniasis vectors in the Kaleybar focus, East Azarbaijan Province, Modarres. J Med Science. 3(1): 9–14.

Rassi Y, Hanafi-Bojd AA (2006) phlebotomine sand flies, vectors of Leishmaniases: Morphology, biology, ecology, and field and laboratory methods with pictorial key of Iranian sand flies. Noavaran-Elm Publication, Tehran, Iran. (In Persian).

Rassi Y, Javadian E, Nadim A, zaherri A, Vatandoost H, Motazedian MH (2005) *Phlebotomus* (*Larrossius*) *kandelakii* the principle and proven vector of visceral Leishmaniasis in northwest of Iran. Pak J Biol Sci. 8: 180–1806.

Rassi Y, Kavirizadeh F, Javadian E, Mohebali M (2004) First report on natural promastigote infection of *Phlebotomus caucasicus* in a new focus of visceral leishmaniasis in northwest of Iran. Iran J Publ Health. 4: 70–72.

Tarallo VD (2010) *Phlebotomine* sand fly population dynamic in a leishmaniasis endemic peri-urban area in southern Italy. Acta Tropical. 116(3): 227–234.

TDR (2008) Research to support the elimination of visceral leishmaniasis, Annual progress Report, Version for JCB. 31.

Triplehorn CA, Johnson NF (2005) Borror and Delong′s Introduction to the study of Insects (7th Ed). Brooks/ Thomson Cole USA. Ser, 701.

WHO (1994) Manual on visceral leishmaniasis control, who/leish/96. 40: 1–79.

Young DG, Duncon MA (1994) Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the west indies, central and south America (Diptera: Psychodidae). Associate Publishers, Gainesville FL.

http://jad.tums.ac.ir
Published Online: January 06, 2016