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Phenology and population dynamics of sand flies in a new focus of visceral leishmaniasis in Eastern Azarbaijan Province, North western of Iran

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ABSTRACT

Objective: To investigate species composition, density, accumulated degree-day and diversity of sand flies during April to October 2010 in Azarshahr district, a new focus of visceral leishmaniasis in north western Iran. **Methods:** Sand flies were collected using sticky traps biweekly and were stored in 96% ethanol. All specimens were mounted in Puri´s medium for species identification using valid keys of sandflies. The density was calculated by the formula: number of specimens/m^2 of sticky traps and number of specimens/ number of traps. Degree-day was calculated as follows: (Maximum temperature + Minimum temperature)/2 — Minimum threshold. Diversity indices of the collected sand flies within different villages were estimated by the Shannon- weaver formula ($H'=\sum_{i=1}^{s} Pi \log_e Pi$). **Results:** Totally 5 557 specimens comprising 16 Species (14 *Phlebotomus*, and 2 *Sergentomyia*) were indentified. The activity of the species extended from April to October. Common sand-flies in resting places were *Phlebotomus papatasi*, *Phlebotomus sergenti* and *Phlebotomus mongolensis*. The monthly average density was 37.6, 41.1, 40.23, 30.38 and 30.67 for Almalodash, Jaragil, Segaiesh, Amirdizaj and Germezgol villages, respectively. Accumulated degree-day from early January to late May was approximately 289 degree days. The minimum threshold temperature for calculating of accumulated degree-day was 17.32 ℃. According on the Shannon-weaver (H´), diversity of sand flies within area study were estimated as 0.917, 1.867, 1.339, 1.673, and 1.562 in Almalodash, Jaragil, Segaiesh, Amirdizaj and Germezgol villages, respectively. **Conclusions:** This study is the first detailed research in terms of species composition, density, accumulated degree-day and diversity of sand flies in an endemic focus of visceral leishamaniasis in Azarshahr district. The population dynamics of sand flies in Azarshahr district were greatly affected by climatic factors. According to this study the highest activity of the collected sand fly species occurs at the teritary week of August. It could help health authorities to predicate period of maximum risk of visceral leishamaniasis transmission and implement control program.

1. Introduction

There are aproximately 700 species of phlebotomine sand flies in 6 genera, of which only two, *ie. Phlebotomus* in the old world and *Lutzomyia* in the new world are medically importance[1]. In the old world, about 40 species of phlebotomus have been proven or suspected vectors of *Leishmania* spp[2,3].

Globally, leishmaniasis is prevalent in 88 countries and affects estimated 12 million people with approximately two million new cases per year, of which 500 000 are visceral leishmaniasis and 1 500 000 are cutaneous leshmaniasis (90% of them in Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Sudan) [4,5].

Since the first report of Pouya in 1949, about 9 000 cases

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of visceral leishmaniasis has been registered in Iran[6]. Although visceral leishmaniasis is seen sporadically throughout Iran, there are four important endemic foci: Ardebil and East-Azerbaijan in the northwest, Fars and Bushehr provinces in the south[7,8]. Recent reports indicate an increasing of new human cases of visceral leishmaniasis as well as new foci of disease in east Azarbaijan, notrth western Iran[9-11]. Some rural areas of Azarshahr district in East Azarbaijan province have been reported to be endemic for visceral leishmaniasis with more than 34 new cases[10,11]. However, wild and domestic carnivores are commonly considered main reservoirs. Rodents have also been considered as reservoirs in the Azarshahr district and 5.35 of them were shown to be seropositive[12].

The geographical distribution and development of insect vectors are strongly related to the climate factors such as temperature, rainfall and humidity. Temperature plays a prominent role in insects' development. It has a direct correlation with the insects, metabolic rates, egg production, survival of pre imaginal stages, adults' longevity and frequency of blood feeding. On the other hand, climate factors have a direct impact on the development of the insects as well as pathogens[13,14].

Phenology is the study of relationships between the weather and biological process such as insect development. The minimum temperature at which insect first start to develop is called the lower developmental threshold and the maximum temperature, at which insects stop developing is called the upper developmental threshold. One degree day is one day (24 hours) with the temperature above the lower developmental threshold by one degree.

Accumulated degree - day is useful in timing scouting events such as when to place traps, when to sample, peak activity and *etc*.

Degree-day for a single day is calculated as follows: maximum temperature+minimum temperature/2—minimum threshold = Degree-day.

The thermal requirements of *Phlebotomus papatasi* (*P. papatasi*) under laboratory conditions were used as a model to match and calculate the thermal requirements of the field sand flies.

The lower developmental threshold temperature for sand flies is 11.60, 19.81, 17.63 and 20.25 ℃ for egg, larva, pupa and pre-oviposition stages, respectively[15].

The mean duration of development days in sand flies are 6, 25, 17, 48, 5-19, 53-63 for egg, larva, pupa, egg to adult, pre- oviposition and egg to egg, respectively[16].

This study is firstly attempt to determine of species richness, relative abundance (diversity), distribution, accumulated degree day requirement for adult emergence of sand flies in a new focus of visceral leishamaniasis in Azarshahr district, east Azarbaijan province, northwestern Iran.

2. Material and methods

2.1. Area study

The investigation was carried out from late April to late October, 2010 in five villages of Almalodash, Jaragil, Segaiesh, Amirdizaj and Germezgol in Azarshahr district (37°30′, 45°40′ E), Eastern Azarbaijan province, northwestern Iran.

The mean annual precipitation is 303 mm with 49% relative humidity, and the mean annual temperature is 15.25 ℃ .

The maximum and minimum average monthly temperatures are 27.7 ℃ and 3.7 ℃, respectively.

The total population of Azarshahr was approximately 103 952 in 2010. The main occupation of the population are farming and raising animals. On the basis of available epidemiologic data obtained from Health centers in Azarshahr district, all villages were selected attentive to having human cases of visceral leishmaniasis.

2.2. Sand flies collection and identification

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 (70 papers per village) were installed at sunset, and collected before sunrise during late April to late October 2010[17,18].

Collected sand flies were stored in 96% ethanol. All specimens with the head and abdominal terminalia cut off were mounted in a drop of Puri's medium for species identification using valid keys of sand flies[7].

2.3. Data analysis

The density of sand flies collected by sticky traps was calculated using this 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, average monthly temperature and average monthly relative humidity in the study area using SAS software[19,20].

The density of species was corrected according to the formula:

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

In order to reduce the dispersion of the variable without modifying the relative position of the species in the multidimensional space[21], species diversity composed of two factors, species richness and species evenness was used. There are many kind of species diversity indexes, a common one is the Shannon-weaver index:

$$H'=\sum_{i=1}^{s} Pi \log_e Pi$$

H′=The diversity index

Pi =The proportion of the ith species

Log_e=Natural log

S=Number of species in the community[22].

Also in order to determine accumulated degree day, records of atmospheric temperature (℃) for the year of 2010 were retrieved from metrology station of the region. The maximum and minimum temperature of each day and

the development zero (minimum or lower threshold) of *P. papatasi* were used to calculate accumulated degree day of field sand flies using the online software accessible at the website of the university of California agricultural and natural resources, and using horizontal cut-off method and single triangle model described by zalom *et al*[23].

There were six possible relationship between daily temperature cycle and the upper and lower developmental threshold. The temperature cycle could be: 1 Completely above both thresholds; 2 Completely below both threshold; 3 Entirely between both threshold; 4 Intercepted by the lower threshold; 5 Intercepted by the upper threshold; 6 Intercepted by the both threshold.

Different equations are required to approximately compute degree days for each case. The relationships between the maximum and minimum temperatures and the development thresholds are used to select the proper equation.

In general, degree days can be calculated using simple formula for average daily temperature calculated from the daily maximum and minimum temperatures, minus the baseline (lower developmental threshold):
(Max Temp+Min Temp)/2－Min Threshold. But in this investigation the single triangle model was used[24].

3. Results

Totally 5 557 specimens comprising 16 species (14 *Phlebotomus* and 2 *Sergentomyia*) were identified including: *P. papatasi* (31.9%), *Phlebotomus sergenti* (*P. sergenti*) (22.5%), *Phlebotomus mongolensis* (*P. mongolensis*) (17.3%), *Phlebotomus ansarii* (*P. ansarii*) (0.3%), *Phlebotomus tobbi* (*P. tobbi*) (3.4%), *Phlebotomus kanelakii* (*P. kanelakii*) (4.2%), *Phlebotomus halepensis* (*P. halepensis*) (8.1%), *Phlebotomus longiductus* (*P. longiductus*) (0.8%), *Phlebotomus anderjevi* (*P. anderjevi*) (2.9%), *Phlebotomus caucasicus* (*P. caucasicus*) (5.0%), *Phlebotomus mesghali* (*P. mesghali*) (0.5%), *Phlebotomus perfiliewi* (*P. perfiliewi*) (0.5%), *Phlebotomus major* (*P. major*) (0.5%), *Phlebotomus* (Adlerius) spp (0.5%), *Sergentomyia hodgsoni* (*S. hodgsoni*) (1.3%), *Sergentomyia pawlowskyi*(*S. pawlowskyi*) (0.3%) (Table 1) .

The activity of the species extended from April to October with a single peak in August. Common specimens in resting places were *P. papatasi*, *P. sergenti* and *P. mongolensis* (Figure 1).

According to formula, monthly average density, number of specimens/m^2 of sticky traps were 37.60, 41.10, 40.23, 30.38 and 30.67 in Almalodash, Jaragil, Segaiesh, Amirdizaj and Germezgol, respectively .

Corrected monthly average density, number of specimens/number of traps, in mentioned villages were 1.47, 1.51, 1.50, 1.39 and 1.40.

Field survery on the population density of sand flies in the region showed that the first adult population occurred in late May and the highest population density was observed at the teritary week of August, followed by a sharp decrease at the first week of October. The accumulated degree day from early January to late May 2010 was approximately 289 degree days when the minimum threshold temperature was 17.32 ℃. It seems the Larvae during diapause (perhaps Ⅳ instar) need 190 degree days to complete their development to pupae at the early spring. Afterwards they could received enough accumulated degree day (113 degree days) to convert to adult in late May when the average temperature was 18.20℃ (Figure 1).

This is similar to the accumulated degree day (297 degree days) necessary for development of *P. papatasi* larvae and pupae under the laboratory condition.

During late May to the second week of October, there was 594 degree days in the studied region and the minimum threshold temperature was considered as 20.25, 11.60 and 19.81 ℃ for development of pre-oviposition, egg, and larvae, respectively.

This condition is adequate for emergence of adults of the first generation in the region. The first generation females are ready to oviposit in mid August to second week of October.

The mean temperature in the early October to early September was 17.20℃, so it was sufficient for hatching of eggs and emerging of second generation′s larvae (considering minimum threshold equal to 11.60 and K is 86.4 degree days).

According to mean temperature (17.40 ℃) in September, there wasn′t sufficient accumulated degree days for successful development of all four larval stages, while the minimum threshold was 19.81℃ and it requires at least 154.75 degree days. This means that sand flies in Azarshahr district have only one generation per year (univoltine). Based on Shannon weaver H′ the diversity of sand flies in studied villages was 0.917, 1.867, 1.339, 1.673 and 1.562 in Almalodasht, Jaragil, Segaiesh, Amirdizaj and Germezgol, respectively. These results showed there were more diversity among sand flies of Jaragil village comparing with other villages.

Table 1
Species composition of sand flies in Azarshahr district, Eastern Azarbaijan, Iran, 2010.

Species	Rf.	*N*
P. papatasi	0.319	1 773
P. sergenti	0.255	1 250
P. mongolensis	0.173	961
P. ansari	0.003	17
P. tobbi	0.034	189
P. kandelakii	0.042	233
P. halepensis	0.081	450
P. longiductus	0.008	44
P. anderjevi	0.029	161
P. caucasicus	0.050	278
P. mesghali	0.005	28
P. perfiliewi	0.005	28
P. major	0.005	28
Phlebotomus (Adlerius) spp	0.005	28
S. sodgsoni	0.013	72
S. pawlowskyi	0.003	17

Table 2
Diversity of sand flies in different villages of Azarshahr district, Northwestern Iran, 2010.

Community	Species	Number	Proportion (Pi)	Log_e Pi	Pi Log_e pi
Community A (Almalodash)	*P . papatasi*	332	0.75	– 2.890	– 0.216
	P. sergenti	17	0.04	– 3.219	– 0.129
	P. tobbi	6	0.01	– 4.606	– 0.460
	P . halepensis	52	0.13	– 2.041	– 0.265
	P . longidactus	12	0.03	– 3.507	– 0.105
	P . perfiliewi	11	0.02	– 3.913	– 0.78
	P . (Adlerius) spp	11	0.02	– 3.913	– 0.78
Community B (Segaiesh)	*P . papatasi*	22	0.21	– 1.560	– 0.328
	P . tobbi	20	0.20	– 1.609	– 0.322
	P . kandelaki	40	0.39	– 0.942	– 0.367
	P . (Adlerius) spp	20	0.20	– 1.609	– 0/322
Community C (Jaragil)	*P . papatasi*	87	0.17	– 1.772	– 0.301
	P . sergenti	100	0.20	– 1.609	– 0.322
	P . mongolensis	109	0.21	– 1.561	– 0.328
	P . tobbi	22	0.05	– 2.996	– 0.151
	P . kandelaki	21	0.04	– 3.219	– 0.129
	P . halepensis	108	0.21	– 1.561	– 0.328
	P . major	10	0.02	– 3.913	– 0.078
	S . hodgsoni	54	0.10	– 2.303	– 0.230
Community D (Amirdizaj)	*P . papatasi*	117	0.40	– 0.916	– 0.366
	P . sergenti	84	0.28	– 1.273	– 0.356
	P . mongolensis	28	0.09	– 2.408	– 0.220
	P . tobbi	11	0.04	– 3.219	– 0.130
	P . kandelakii	6	0.02	– 0.912	– 0.078
	P . halepensis	11	0.04	– 3.219	– 0.129
	P . anderjevi	22	0.07	– 2.660	– 0.186
	P . caucasicus	11	0.04	– 3.219	– 0.130
	P . mesghali	6	0.02	– 3.912	– 0.078
Community E (Germezgol)	*P . sergenti*	67	0.16	– 1.833	– 0.293
	P . mongolensis	137	0.33	– 1.109	– 0.366
	P . anderjevi	68	0.17	– 1.772	– 0.301
	P . halepensis	70	0.17	– 1.772	– 0.301
	P . kandelakii	68	0.17	– 1.772	– 0.301

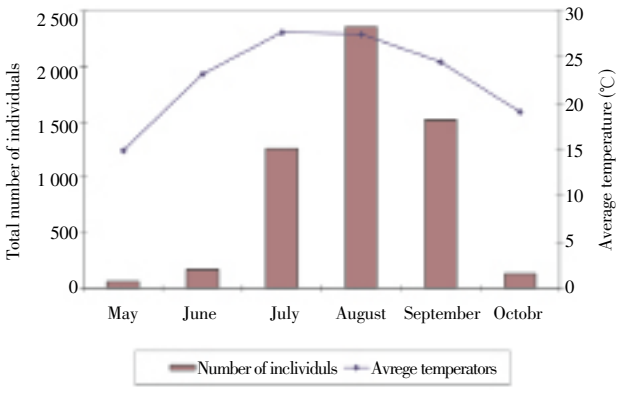

Figure 1. Total number of sand flies collected each month, and variation of monthly mean temperature, Azarshahr district, eastern Azarbaijan , Iran , May – October 2010.

4. Discussion

It is reported *Leishmania* vectors are available in the north west of Iran. Although visceral leishamaniasis occurs sporadically throughout Iran, the disease is endemic in several parts of northwestern Iran. Three sand fly species, *P. perfiliewi transcaucasicus*, *P.* (*Larroussius*) *kandelakii* Shchurenkova and *P. (Larroussius) major* Annandale are proven vectors in Iran[7, 9, 25]. Investigations on vectors of VL in Ardebil and eastern Azarbaijan provinces showed two species of *P. kandelakii*, *P. perfiliewi* were the vectors of the disease[26–28].

Two other species, *P. (Larroussius) keshishiani* Shchurenkova, *Phlebotomus (Paraphlebotomus) alexandri* Sinton have been found naturally infected with promastigotes and are suspected vectors of visceral leishamaniasis in the country[29,30]. According to former findings and our

collection, it seems *P. kandelakii* and *P. perfiliewi* are two probable vectors of visceral leishamaniasis in the region.

Control of leishmaniasis depends on ecological and epidemiological information pertaining to the disease, such as identification of preferred hosts, detection of natural infections in the vector(s), phenology of vectors and estimating the population density of vectors insects.

Degree day is a useful tool for estimating the population density in the field[31]. Models based on population dynamics and environmental parameters facilitate the prediction of the number of generation per year, the potential ability of population increase, and distribution along temperature gradients[32-41].

We demonstrated that the predictive degree day model is a useful tool for estimating the development, population density, and number of generations of sand flies in the field. This model provides valuable information about temporal and spatial distribution of vectors of visceral leishamaniasis in the region. Our study showed that the activity of sand flies is started in the end of May and extended until early October with a single peak in August. This study indicated that optimal and suitable humidity and temperatures were 30%-40% and 25-30 ℃, respectively in the region, while the epidemiological data reported by health authorities in the region showed that the visceral leishamaniasis cases occurred in August-September on wards. Studies of Rioux *et al* showed that the optimum temperature for the development of *Leishmania infantum* in sand flies was circa 25 ℃ and compatible with high density of sand flies in August. Higher temperature not only accelerates the development of sand fly vector, but also shortens the development times of *Leishmania* parasite in vectors[42].

In late May as well as early October the mentioned parameters were 40%-70% and 15-20 ℃ with very low density of sand flies. Knowledge of spatial and seasonal trends of sand flies will help decide both where and when to implement control programs[43]. The length of the activity period and sand fly densities are largely regulated by climate conditions that affect their life cycle. The minimum threshold temperatures for egg, larvae, pupae, and total development were 11.60, 19.80, 17.63 and 20.25 ℃, respectively[15].

The result of this study showed, ranges of adult emergence were between 11.0-25.4 ℃ and increased rapidly with adding of temperature. The highest population density was observed in tertiary week of August, but there was rapidly decreasing of sand flies in second week of October with complete disappearing of them in tertiary week of October.

This study is the first detailed research in terms of species composition, density, accumulated degree-day and diversity of sand flies in an endemic focus of visceral leishmaniasis in Azarshahr district and showed the population dynamics of sand flies were greatly affected by climate factors in the region.

To sum up, our results suggest that the highest activity of the collected sand fly species occurs in tertiary week of August, that could help health authorities for prediction period of maximum risk of visceral leishamaniasis transmission and successful implementation of a control program.

Conflict of interest statement

We declare that we have no conflict of interest.

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