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Highlights

- Papatasi fever, or three-day fever, is transmitted by the female sandfly species *Phlebotomus papatasi*, with symptoms resembling influenza. Robat Karim County's climate and high sandfly population create an ideal environment for *P. papatasi*. The study aims to conduct molecular monitoring of the Papatasi fever virus in the sandflies of this region.
- In Robat Karim County, 679 sandflies were collected using traps and aspirators across seven selected areas. A random selection of 500 samples was transferred to the Medical Entomology Department laboratory. Species identification and frequency were determined, followed by molecular examination for Papatasi virus using Real-time PCR at Tehran Pasteur Institute.
- Out of the 679 sandfly samples, 310 (45.65%) were males and 369 (54.35%) were females. Seven species were identified, including three species of the genus *Phlebotomus* and four of the genus *Sergentomyia*, with *Phlebotomus* sergenti being the most prevalent (28.57%). None of the 500 samples tested for Papatasi virus infection via Real-time PCR were positive.
- Robat Karim is an endemic area for infectious diseases such as leishmaniasis. Climate change in the region favors the growth and reproduction of *Phlebotomus papatasi*, a vector for sandfly fever. Since there is no vaccine for sandfly fever, preventive measures are crucial for controlling and reducing sandfly-transmitted diseases.

Abstract: Introduction: Papatasi fever, or three-day fever, is a viral disease transmitted by a species of female sandfly called *Phlebotomus papatasi* that has symptoms similar to influenza. Robat Karim County, due to the high abundance of Sandfly and climate change in recent years, provides a suitable environment for the growth and reproduction of Sandfly, especially *Phlebotomus papatasi* Therefore, the aim of this study was molecular monitoring of Papatasi fever virus in Sandfly of Robat Karim County, Tehran province. **Materials and methods:** In 7 selected areas in Robat Karim County, 679 Sandfly using sampling methods including traps and aspirators were caught and 500 samples were randomly selected according to the fishing location and transferred to the laboratory of the Medical Entomology Department. After fixing Sandfly on the slide surface, identification and percentage of frequency of sandfly species were determined and then by Real-time PCR method, molecular examination of Papatasi virus infection was performed at Tehran Pasteur Institute. **Findings:** Out of 679 samples taken, 310 (45.65%) belonged to males and 369 (54.35%) belonged to females. 7 species including 3 species of Sandfly of the genus *Phlebotomus* and 4 species of the genus *Sergentomyia* were identified among the samples, the highest percentage of catch belonged to *Phlebotomus sergenti* (28.57%). Of the 500 samples sent to the laboratory for testing for *phleboviruses* by Real-time PCR, no positive cases of Papatasi virus infection were observed in them. **Conclusion:** Robat Karim is one of the endemic areas of infectious diseases such as leishmaniasis. Due to climate change in this region that conditions for the growth and reproduction of Sandfly, especially *Phlebotomus papatasi* as a Vector of sandfly fever and since the sandfly fever disease is not vaccinated therefore, preventive measures to control and reduce diseases transmitted by Sandfly are important.

Introduction

Today, arbovirus diseases are considered as one of the most important diseases transmitted by arthropods and have caused concerns in the health debate [1,2]. Three-day fever is a viral disease transmitted by Sandfly and Symptoms similar to influenza such as headache, high fever, back pain when moving the eye, conjunctival hyperemia, contusion, lethargy, nausea and decreased white blood cell count [3,4].The cause of three-day fever is transmitted by *Phlebotomus* of phleboviruses (family of psychodia) [5,6]. Phleboviruses belong to the phenoviride family. There are 53 regions of viral antigens in the genus Phleboviruses, and 9 species have been defined by the International Report of the Committee for Classification of Viruses [7,8]. Sandfly carry leishmaniasis, Three-day fever and carrion disease and are important [9,10]. The main Vector of this disease is *Phlebotomus papatasi*, which is present in different parts of the world and is the cause of the disease in humans [10–12]. Iran, characterized by diverse climatic conditions ranging from arid deserts to humid coastal areas, provides a conducive environment for various species of sandfly. The most significant Sandfly species in Iran, with respect to disease transmission, include Phlebotomus papatasi and Phlebotomus sergenti. Northern Regions: The Caspian Sea littoral, encompassing provinces like Mazandaran and Gilan, exhibits a high prevalence of Phlebotomus species due to its humid climate. Central and Southern Regions: Desert and semidesert areas, such as those in the Yazd, Kerman, and Fars provinces, also support substantial Sandfly populations. Western and Eastern Regions: The mountainous and highland areas, including Kurdistan and Sistan and Baluchestan, respectively, present moderate to high densities of Sandfly populations. Seasonal Distribution: Sandfly activity in Iran is highly seasonal, with peak populations typically observed from late spring to early autumn. Central and Southern Iran: These regions report a higher incidence of Papatasi fever, correlating with the high density of Phlebotomus papatasi. Western and Eastern Iran: There is a lower but notable incidence of Papatasi fever. Vector Control and Prevention Strategies Controlling the spread of sand flies and subsequently Papatasi fever involves a multifaceted approach: Environmental Management: Reducing breeding sites through proper waste management and drainage improvements. Public Health Education: Increasing awareness about Sandfly habitats, peak activity periods, and preventive measures. The prevalence of Papatasi fever is closely linked to the density and activity patterns of Sandfly populations [13,14]. and on the other hand, unaware of the status of this disease and its Vector phleboviruses, so this study was performed for molecular monitoring of Papatasi fever virus. Soil mosquitoes were carried out in Robat Karim County, Tehran province in 2023.

Materials and Methods

Sampling Location and Method of Sandfly Hunting

Initially, a detailed study was conducted on the distribution rate and activity season of Sandfly in the study areas in Robat Karim County. In this study, sticky traps, light traps with normal light (UV light) and an aspirator were used to hunt Sandfly. Sandfly hunting operations were carried out from the second half of March to the end of November. The captured samples were transferred to acetone-containing tubes for degreasing for several seconds. After a few minutes, 96% alcohol was added to the samples and then kept in the laboratory for identification and determination of species and refrigerated at 4 °C. The number of Sandfly was recorded by habitat (human and animal) and fishing places (indoor and outdoor), fishing date, Sex of Sandfly and their abdominal status.

Number of Samples and Identify Sandfly

For this study, about 679 Sandfly of different species were hunt, and 500 selected samples were randomly transferred to the laboratory of the Department of Medical Entomology according to the

location of the catch. The collected samples were poured into a petri dish and using a drop of purulent medium on the slide and transfer of Sandfly to the slides, the Sandfly were isolated and fixed. Depending on the type of sandfly caught, the placement on the slide was different. For *Phlebotomus*, upward and for *sergentomyia*, the ventral surface of the head was up and the tentacles were down. Place the fixed slide on a wooden rail at room temperature for 24-48 hours to dry. In order to determine the identity and morphological characteristics of Sandfly, including cyberium teeth and temperaments, the species were studied and then the species were identified with a valid key to identify Iranian Sandfly [15,16]. Date, place and time of fishing were recorded along with genus and species of Sandfly. The results of identification as well as the abundance and dispersion of samples in each region were recorded.
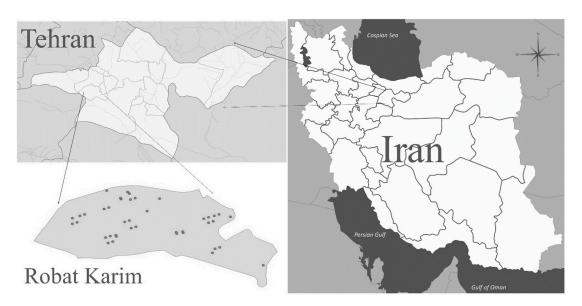

Figure 1. Sampling locations in Rabat Karim city.

Method of Identification and Isolation of Papatasi Fever Virus

Light traps and aspirators were used to identify and isolate Papatasi fever virus from soil mosquitoes. Immediately after catching, the mosquitoes were placed in tubes containing RNA LATER for molecular testing and evaluation of Papatasi fever virus in the transfer medium until extraction at minus 70 degrees.

Real-Time PCR

RNA extraction was performed using Qiagen Company kit instructions and Cat No./ID:85300 and according to the protocol used in the Arbovirology Department of Pasteur Institute of Tehran**.** In order to identify Papatasi fever virus, Nested RT-PCR technique was used One-Step RT-PCR kits for the first PCR and TaKaRa Ex Tag® kit (Mg2 + free Buffer) for the second PCR. Temperature and time program was considered for Real Time RT-PCR steps**.** To design the primer and identify the virus genome, sequences similar to the virus genome and different serotypes were extracted from the NCBI site. Then we started alignment using mega 6 Gene Runner 4.0 software and finally the primer was designed based on the protected points. Finally, gene expression analysis was done by Real-time device.

Statistical Analysis

Using the estimated sample size using the following formula, about 322 Sandfly were required and more (about 500 samples) were selected to be sent to the laboratory.

$$n = \frac{2 \times (Z1_{-\frac{\alpha}{2}} + Z1-\beta)2[P(1-P)}{(P1-P2)2}$$

SPSS software version 22 was used to analyse the data of this study, frequency distribution tables, calculation of central indices, mean and calculation of scatter indices (standard deviation).

Result

Finds of Sandfly Fauna

679 Sandfly during 7 months (May, June, July, August, September, October and November) during spring, summer and autumn from 7 regions located in Robat Karim, including: Emamzadeh Abu Taleb, Manjil Abad, Vahn Abad, Hamedanak, Esmail Abad, Saleh Abad and Meymanat Abad was identified. The results showed that the activity of Sandfly in domestic and Outdoor places started in early May and ended with 2 peak activities (one in July and the other in September), in November and the highest percentage of Sandfly catch was reported for Human habitat (62.88%) and indoor places (216) which showed a high abundance of Sandfly in these places (Table 1). Out of 679 samples of caught Sandfly, Vahn Abad region had the highest percentage of abundance of caught Sandfly (149 = 21.94%). The results of relative abundance of Sandfly by type of human and animal catch (indoor and outdoor) showed the abundance of Sandfly caught in Outdoor places (50.36%). Of the 679 samples taken, 310 (45.65%) were male and 369 (54.35%) were female. The study of sex ratio (310 males versus 369 females) showed that males are not the dominant species in this region and the highest number belongs to females (Table 1).

Results of Identified Species

After identifying the samples, using a valid key to identify Iranian Sandfly, species were identified in the laboratory under a microscope. Female sex organs (Spermatheca) (Figure 2) and male sex organs (genitalia) (Figure 3) were identified in Sandfly under a microscope after fixation.

Species of Caught Sandfly

7 species including 3 species of *Phlebotomus* Sandfly and 4 species of *Sergentomyia* were identified among the samples. The identified species belonged to the genus *Phlebotomus* including: *Phlebotomus papatasi*, *Phlebotomus alexanderi* and *Phlebotomus ansarri*. Also identified species belonging to the genus *Sergentomyia* are: *Sergenotomyia antennata*, *Sergentomyia sintoni* and *Sergentomyia clydei*. *Phlebotomus papatasi* and *Phlebotomus Ansari* species had the highest and lowest percentages, respectively.

Results of Sandfly Infection with Phleboviruses

The results of Real-time PCR showed that out of a total of 500 samples sent to the laboratory, no positive cases of Papatasi virus infection were observed in the study areas.

Table 1. Sandfly fauna caught according to habitat and fishing location in Robat Karim County, 2023.

No.	Parts	Sites	Species	Number	Pi	Log Pi	Pi (Log Pi) (Pi Ln)	Gender		Human		Animal	
								Male	Female	Indoor Places	Outdoor Places	Indoor Places	Outdoor Places
1		*Emamzadeh Abu Taleb* N=94 Shannon=1.715 Evenness=0.881 Simpson=0.205 1 – D =0.795	*P. papatasi*	34	0.361	1.018	0.367	18	16	4	17	4	9
			P. sergenti	13	0.138	1.980	0.273	10	3	2	8	3	0
			P. alexandri	18	0.191	1.655	0.316	7	11	7	7	3	1
			S. sintoni	5	0.053	2.937	0.155	2	3	0	4	1	0
			S. dentata	8	0.085	2.465	0.209	7	1	2	3	2	1
			S. baghdadis	12	0.127	2.063	0.262	4	8	1	4	3	4
			S. antennata	4	0.042	3.170	0.133	3	1	0	2	0	2
2		*Manjil Abad* N=104 Shannon=1.627 Evenness=0.908 Simpson=0.207 1 – D =0.793	*P. papatasi*	28	0.269	1.313	0.353	9	19	5	12	7	4
			P. sergenti	30	0.288	1.244	0.358	21	9	11	8	6	5
			P. alexandri	17	0.163	1.814	0.295	3	14	3	8	2	4
			S. sintoni	4	0.038	3.270	0.124	0	4	0	2	0	2
			S. dentata	16	0.153	1.877	0.287	11	5	3	5	2	6
			S. baghdadis	9	0.086	2.453	0.210	2	7	0	6	0	3
			S. antennata	0	0	0	0	0	0	0	0	0	0

3	Central	**Vahn Abad** N=149 Shannon=1.443 Evenness=0.581 Simpson=0.259 1 – D =0.741	*P. papatasi*	36	0.241	1.422	0.342	9	27	12	9	6	9
			P. sergenti	41	0.275	1.290	0.354	16	25	20	6	10	5
			P. alexandri	52	0.348	1.055	0.367	33	19	24	13	3	12
			S. sintoni	13	0.087	2.441	0.212	2	11	4	3	2	4
			S. dentata	0	0	0	0	0	0	0	0	0	0
			S. baghdadis	2	0.013	4.342	0.056	0	2	0	1	0	1
			S. antennata	5	0.033	3.411	0.112	3	2	0	2	3	0
4		**Hamedanak** N=74 Shannon=1.506 Evenness=0.840 Simpson=0.219 1 – D =0.781	*P. papatasi*	17	0.229	1.207	0.276	12	5	6	2	4	5
			P. sergenti	23	0.310	1.171	0.363	14	9	8	4	10	1
			P. alexandri	10	0.135	2.002	0.270	1	9	1	5	2	2
			S. sintoni	18	0.243	1.414	0.343	6	12	7	4	5	2
			S. dentata	4	0.054	2.918	0.157	1	3	0	1	3	0
			S. baghdadis	0	0	0	0	0	0	0	0	0	0
			S. antennata	2	0.027	3.611	0.097	0	2	0	1	0	1
5	Bostan	**Esmail Abad** N=99 Shannon=1.555 Evenness=0.868	*P. papatasi*	8	0.080	2.525	0.202	3	5	1	2	1	4
			P. sergenti	35	0.353	1.041	0.367	22	13	7	11	13	4
			P. alexandri	27	0.272	1.301	0.353	6	21	17	4	5	1
			S. sintoni	15	0.151	1.890	0.285	5	10	3	5	1	6

#	Region	Location / Stats	Species										
		Simpson=0.234	*S. dentata*	11	0.111	2.198	0.243	2	9	2	4	0	5
		1 – D =0.766	*S. baghdadis*	0	0	0	0	0	0	0	0	0	0
			S. antennata	3	0.030	3.506	0.105	1	2	0	0	0	3
6		**Saleh Abad**	*P. papatasi*	12	0.164	1.807	0.296	5	7	3	4	0	5
		N=73	*P. sergenti*	19	0.260	1.347	0.350	6	13	11	6	1	1
		Shannon=1.756	*P. alexandri*	15	0.205	1.584	0.324	14	1	6	2	4	3
		Evenness=0.902	*S. sintoni*	5	0.068	2.688	0.182	4	1	0	1	3	1
		Simpson=0.174	*S. dentata*	1	0.013	4.342	0.056	1	0	0	1	0	0
		1 – D =0.826	*S. baghdadis*	8	0.109	2.216	0.241	2	6	0	2	6	0
			S. antennata	13	0.178	1.725	0.307	4	9	3	4	4	2
7	*Golestan*	**Meymanat Abad**	*P. papatasi*	29	0.337	1.087	0.366	13	16	18	9	0	2
		N=86	*P. sergenti*	33	0.383	0.959	0.367	17	16	20	6	2	5
		Shannon=1.386	*P. alexandri*	10	0.116	2.154	0.249	4	6	2	6	0	2
		Evenness=0.861	*S. sintoni*	0	0	0	0	0	0	0	0	0	0
		Simpson=0.279	*S. dentata*	0	0	0	0	0	0	0	0	0	0
		1 – D =0.721	*S. baghdadis*	8	0.093	2.375	0.220	2	6	1	6	0	1
			S. antennata	6	0.069	2.673	0.184	5	1	2	1	0	3
		Total		679				310	369	216	211	121	131

	679	427	252
		679	

** The table from the article titled "**Understanding Sandfly Vector Dynamics: Molecular Surveillance of Papatasi Fever Virus in Robat Karim County, Tehran**" presents detailed data on the distribution and characteristics of sandfly species across different sites within Robat Karim County. The data is broken down into several columns, each providing specific information about the sandfly species identified | at each site. Here is a detailed interpretation of the table.

Site Information and Diversity Indices

Sites: The locations surveyed are Emamzadeh Abu Taleb, Manjil Abad, Vahn Abad, Bostan Hamedanak, Esmail Abad, Golestan Saleh Abad, and Meymanat Abad.

N: The total number of sandflies collected at each site.

Shannon Index: A measure of species diversity; higher values indicate greater diversity.

Evenness: Reflects how evenly the individuals are distributed across the species; values close to 1 suggest a more even distribution.

Simpson Index (D): Measures the probability that two individuals randomly selected from a sample will belong to the same species; lower values indicate higher diversity.

1 - D: A complementary measure to the Simpson Index, indicating the diversity more intuitively where higher values suggest higher diversity.

Species-Specific Information

Species: Various sandfly species identified, including Phlebotomus papatasi, P. sergenti, P. alexandri, Sergentomyia sintoni, S. dentata, S. baghdadis, and S. antennata.

Number: The count of each species at each site.

Pi: Proportion of each species in the total sandfly population at the site.

Log Pi: Natural logarithm of Pi, indicating the frequency distribution.

Pi (Log Pi): Product of Pi and its logarithm, used in diversity calculations.

Gender and Location Distribution

Gender: Distribution of males and females for each species.

Human/Animal Indoor/Outdoor: Indicates the number of sandflies found in different environments—human indoor places, human outdoor places, animal indoor places, and animal outdoor places.

Discussion

Key Observations

Species Distribution: *P. papatasi and P. sergenti* are generally the most abundant species across all sites. *P. alexandri* shows significant presence in some sites like Vahn Abad. *S. sintoni* and *S. dentata* are less common but present in certain locations.

Diversity Indices

Shannon and Simpson indices vary across sites, indicating different levels of species diversity.

Golestan Saleh Abad has the highest Shannon index (1.756), indicating the greatest species diversity, while Meymanat Abad has the lowest (1.386).

Evenness

Evenness values are generally high, indicating a relatively balanced distribution of species at most sites.

Gender Distribution

In most sites, there is a mix of male and female sandflies, though certain species may have more pronounced gender distributions.

Environmental Preferences

Sandflies are found in various environments, with some species showing preferences for indoor or outdoor, human or animal environments. Table 1. provides valuable insights into the ecology and

distribution of sandflies in Robat Karim County, which can inform vector control strategies and public health interventions for diseases like Papatasi fever.

The results of the study in Jask city showed two peaks of sandfly activity in May to June and October to November [16].The results of the study in Kalaleh County showed two peaks of sandfly activity in July and September [17].In this study, it was also shown that Sandfly had 2 activity peaks (in July and in September).In a study showed that male Sandfly were the predominant species in their target areas[18].According to previous studies, male Sandfly are caught early in the maturing season and female Sandfly are caught in the late maturing season[19,20]..In the present study, it was shown that out of a total of 679 samples, 310 (45.65%) were male and 369 (54.35%) were female. The study of sex ratio (310 males versus 369 females) showed that males are not the dominant species in this region, and the highest number belongs to females. Also, 9 species of phlebotomus and 12 species of sergentomyia have been identified in the study of soil mosquito fauna of Khash city[21]. In the present study, 7 species including 3 species of *Phlebotomus* and 4 species of *Sergenotomy* were identified. the highest abundance of Sandfly in indoor and outdoor places belonged to *P. papatasi*, which was consistent with other studies conducted in different parts of the country. Other species identified are *P. alexanderi* and *P. ansarri*. Also identified species belonged to the genus *Sergentomyia* including *S. antennata*, *S. clydei* and *S. syntoni*. *P. ansarri* was not found in previous studies, which differed from the findings of the present study. Previous studies of Phleboviruses in Sandfly and rodents have shown viral infection in these specimens[22,23].The first report of Corfu virus in four species of Sandfly in Iran was detected by Real Time RT-PCR [24].In the present study, no positive case of Papatasi virus infection was found in molecular studies.

Conclusion

The results of this study showed that Sandfly, especially Phlebotomus species, are widely distributed in the region and Robat Karim is one of the sporadic areas of infectious diseases such as leishmaniasis. Due to climate change in this region, which provides the conditions for the growth and reproduction of Sandfly, especially Phlebotomus papatasi as a vector of sandfly fever. Since mosquito fever is not vaccinated, preventive measures should be taken to control and reduce mosquito-borne diseases.

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