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Diversity and Activity Pattern of Sand Flies in Cutaneous Leishmaniasis Endemic Tribal District Khyber, Pakistan

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ABSTRACT

Leishmaniasis is endemic for last more than one decade in the tribal district Khyber located in the north-west of Pakistan near Pak-Afghan border. The disease burden has increased during the time of war against terrorism in the tribal belt of Pakistan bordering Afghanistan. This study was designed to investigate various ecological and entomological aspects of sand flies in war effected district Khyber. Sand flies were collected from seven different localities of the district by flit method, CDC light traps and mouth aspirator. A total of 28 species belonging to two genera i.e., *Phlebotomus* represented by 12 species and *Sergentomyia* represented by 16 species were collected during the present study. *Phlebotomus mongolensis* is reported for the first time from Pakistan. *Phlebotomus sergenti* (48.77%) was the most abundant species followed by *Phlebotomus paptasi* (17.89%), *Sergentomyia baghdadis* (14.53%) and *Sergentomyia babu* (7.22%). Shannon-Weiner Index was high due to maximum number of specimens belonging to *Phlebotomus sergenti.* The overall female to male sex ratio was observed to be 1:1.9. Sand fly activity was observed from April to December with population density reaching its peak in the month of August. A positive correlation was observed between sand fly abundance and climatic variables, which was significant with temperature only. The high abundance of *Phlebotomus sergenti* support the endemicity of anthroponotic cutaneous leishmaniasis in the study area. Presence of species of subgenera *Adlerius* and *Larusius* suggest the possibility sporadic visceral leishmaniasis in the region.

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SBR designed the study. MZS collected the samples. MZS and SBR identified the samples, analyzed the data and wrote the manuscript.

Key words
Sand flies, Diversity, Ecology, Seasonal variation, Pakistan

INTRODUCTION

The Phlebotomine sand flies are distributed throughout the tropics and subtropics as well as in the temperate regions of the world. The subfamily Phlebotominae consists of nearly 1000 species classified in six genera, of which the genus *Phlebotomus* have 13 subgenera, *Sergentomyia* have ten subgenera and the genus *Chinius* have four species and are found in the old world. The phlebotomine sand flies are found everywhere and is a group of medically important insects responsible for the transmission of protozoan parasites and some arbo-viral infections among humans and other animals. The role of sand flies as vectors of several viral, bacterial and protozoal diseases emphasizes the significance of investigating and understanding the ecology, diversity and other biological characteristics of these insects. Many species of sand flies act as vectors of leishmaniasis. This disease is mainly present in three forms; cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL) and visceral leishmaniasis (VL) (Jal., 2022). Zoonotic cutaneous leishmaniasis (ZCL) caused by *L. major* and anthroponotic cutaneous leishmaniasis (ACL) caused by *L. tropica* are common in Pakistan. CL is endemic in many countries of Eastern Mediterranean region (Ashford, 1996).

The sand fly fauna of Pakistan is a mixture of Palearctic, Oriental and Afro tropical regions (Lewis, 1967). The topography and climatic conditions of Pakistan provides an ideal breeding and living habitat for various species of sand flies (Lewis, 1978). Several studies have been conducted on sand flies of Pakistan, but still current literature provides insufficient information about the sand fly species diversity of this region (Qutubuddin, 1951). The work of Lewis (1967), investigating the fauna of sand flies in Pakistan and its relation to spread of leishmaniasis, has a leading role. Aslamkhan *et al*. (1998) reviewed literature and carried out a study to investigate the biodiversity of sand flies of Pakistan and reported 29 species in his work. Kakarsulemankhel (2003, 2004) studied the sand flies of Balochistan and updated the sand fly fauna of Pakistan

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comprising of 37 species. Ali *et al*. (2016a) reported 14 species of sand flies from the newly merged Tribal district of North Waziristan. Ali *et al*. (2016b) identified 20 species of sand flies from districts Dir Lower and Upper and ten species of sand flies had been reported from another newly merged Tribal district, Bajaur (Wahid *et al*., 2020).

The incidence of CL is increasing from time to time in the world, mainly due to the unlawful intrusion of people into the forests and natural habitats where the potential vectors of *Leishmania* and reservoir animals are present. Some environmental factors also influence the survival of these insects and they can be found only in areas where conditions are suitable in terms of temperature and relative humidity. CL has become a serious public health problem in Pakistan and is highly endemic in tribal district Khyber for last more than one decade. More than 40000 cases has been reported during 2012-2019 and the causative agent has been identified as *L. tropica* by using PCR (Khan *et al*., 2016). However, data regarding sand fly species, their relative abundance, and seasonal variation is lacking. This study aims at exploring the diversity, ecology, and seasonality of sand flies in anthroponotic CL endemic tribal district Khyber. This knowledge will help in devising an effective strategy for the control of disease in the affected area.

MATERIALS AND METHODS

Historical background of the study area

Khyber Pass is located on one of the most well-known and the most strongly secured mountain ranges on the globe (The Hindukush). It has a universal historical heritage and has made an important position throughout the known history. It is always marked mainly as a corridor, through which almost all the infiltrations to the Indian subcontinent occurred, including those by the Aryans, Alexander, Mahmud of Ghazni, Tamerlane, the Mughals and the Durrani Afghans. When NATO (North Atlantic Treaty Organization) forces under the leadership of United States, attacked Afghanistan in 2001, militants penetrated the tribal district Khyber located at the Pak-Afghan border of Pakistan. Due to the absence of an active security network in the region at that time and its rough, mountainous boundaries and territory, the area became a hotbed of the insurgents. Over 90% of the district came under control of terrorist in 2007 and the area was used by militants to launch attack on civil and military establishments of Khyber Pakhtunkhwa (KP) killing thousands of people and wounding many more. After the military operations of 2012 and 2014, Khyber has been cleared of the militants but it has destroyed the infrastructure of the area and vector borne diseases like ACL has been occurring on epidemic scale since then.

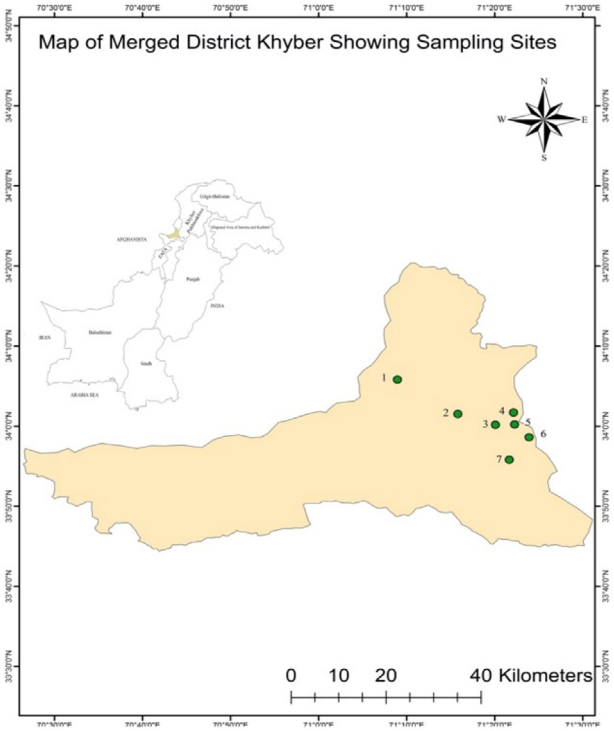

Fig. 1. Map showing sampling sites in the tribal district Khyber, Khyber Pakhtunkhwa. 1, Landi Kotal; 2, Ali Masjid; 3, Sur Kamar; 4, Gudar; 5, Jamrud; 6, Shah Kass; 7, Qambar Khel.

Sampling sites

Khyber district is among the most CL affected areas of Pakistan. Sampling was done from seven villages distributed in all four tehsils of the district. As most of the area of Khyber is covered with mountain ranges, the majority of the population lives along the Khyber Pass and in the plain areas touching district Peshawar and Kohat. The seven sampling sites (Fig. 1) were: (a) Landi Kotal (Tehsil Landi Kotal, altitude = 1072 m above sea level (ASL)), (b) Ali masjid (Tehsil Jamrud, 967 m ASL), (c) Sur Kamar (Tehsil Jamrud, 510 m ASL), (d) Gudar (Tehsil Mulla Gory, 497 m ASL), (e) Jamrud (Tehsil Jamrud, 461 m ASL), (f) Shah Kass (Tehsil Jamrud, 341 m ASL) and (g) Qamber Khel (Tehsil Bara, 450 m ASL). Landi Kotal is a hilly station, Ali Masjid and Sur Kamar lies in the foothills, Gudar, Jamrud and Shah Kass are plain areas in the foot hills, while Qamber Khel consist of plain area. Ali Masjid is located in the foothills. The houses in these areas were of mixed type. In hilly areas special type of wide tunnels are excavated in or near the houses located in the foothills locally called ‘Gara’ which are used in the summer season

for rest. These Garas are used as animal sheds and in some areas used as mixed dwellings. Gara were a rich source of sand fly collection due to microclimate they used to provide for resting sand flies.

Sampling methods

In each collection site, ten to fifteen houses were selected randomly. In each house two rooms comprising of an animal room and a human dwelling were used for the collection of adult sand flies. Each collection site was visited once in a month. Most of the rooms from where collection of sand flies was made were having mud walls and thatched ceilings. The adult sand flies were collected by using flit method, CDC light traps and mouth aspirator.

Flit method and use of aspirator

Resting sand flies from human dwelling and animal rooms were collected by using flit method (Lane and Crosskey, 1993) supplemented by mouth aspirator. Collection was conducted three to four hours after sunrise. In this method, the floor of the room was covered with black sheets (to make the sand flies easily visible with dark background) and all the insects resting inside the room were killed by making the room airtight and then spraying a fast-acting volatile pyrethroid insecticide (Mortein: Reckitt Benckiser Pakistan Ltd.) for 30 sec. For this purpose, all the occupants including animals and articles like tables, chairs, exposed edibles etc., were removed from the room to be sprayed, or appropriately covered. The doors and windows were kept closed and after 10-15 min the room was opened, the sand flies from the sheets were collected with the help of fine entomological forceps and placed in a tube filled with 90% ethanol. Sometimes flitting was followed by sampling live sand flies from the room, using a mouth aspirator. The number of live specimens collected from a site were added to those collected by flitting to calculate the total number of sand flies. Collection from each room was kept in a separate tube and was labeled accordingly.

CDC light traps

The sand flies were also collected by using battery operated CDC miniature light traps (model 512 with air-actuated gate system and LCS-2 photo switch; John W. Hock Company, USA). For collection, a light trap was hanged 30 inches above the ground from dusk to dawn. Sand flies captured by these traps were then transferred to 90% ethanol for further processing.

Sand fly processing

Species identification of sand flies was done based on morphological and anatomical structures which can be exposed by preparing a permanent slide of each fly. For this purpose, the head of the male was separated from the body by using fine entomological needles under a stereo microscope (SD 30, Olympus, Japan). The head was placed dorsally and body laterally, taking care that the wings and legs were properly placed. A drop of Puri's media was put on the head and body before placing the cover slip. For mounting the female, the last three abdominal segments and head were separated from the body as anatomical structures in head and spermatheca were used for identification purpose. These body parts were placed on glass slide and covered with a cover slip after putting a drop of Puri's media. The slides were kept safe for at least three weeks for clearing of the body parts. The sand flies cleared after fixing on the slides were examined individually under a binocular microscope (Nikon E-100, Japan) by using 40X objective and identified up to the species level with the help of valid taxonomic keys illustrated in Artemiev (1976) and Lewis (1982). The slides of identified specimens were catalogued, placed in slide boxes and were kept in the Vector Biology and Entomology Laboratory of Institute of Zoological Sciences, University of Peshawar as reference material.

Data arrangement and analysis

Data regarding species identification was transferred to excel sheet and was arranged according to locality and months. Following ecological indices were calculated for the overall data obtained and each collection site.

Species richness (S) = number of species in the study area.

Relative abundance (p_i) = n/N x 100 where n is the number of specimens of a particular species and N is the total number of sand flies collected from a study area. Three categories of dominance were made on the basis of relative abundance: dominant (D): >10%, subdominant (D): 1-10% and satellite (S): <1%.

Degree of presence (C) = l/L x 100 where l is the number of localities where the species was found and L is the total number of studied localities. Species found in more than 80% localities were classified as constant species (C), those captured in 60.1 to 80% localities were frequent species (F), 40.1 to 60% were classified as moderate (M) species, 20.1 to 40% as infrequent (I) species, while 0 to 20% as sporadic species (S).

Sex ratio (female/male) was calculated for each species collected during the survey.

Shannon-Weiner Index for calculating species diversity (H) = $-\sum p_i \times ln p_i$ where $ln\ p_i$ is natural log of p_i.

Evenness (J or E or Pie-Lou's index) (E) = H/*ln*S where *ln* S is natural log of species richness.

Simpson index (D) =1- $\{[\sum n(n-1)]/N(N-1)\}$

Month wise abundance of sand flies was plotted against climatic variables i.e. temperature, relative humidity and rainfall using excel. Correlation coefficient and its statistical significance ($P<0.05$) was calculated by using online statistical software (Wessa, 2017).

RESULTS

Species composition of sand flies

A total of 11766 sand flies (4015 female and 7751 male) belonging to 28 species of two genera and 11 subgenera were collected as adults from the tribal district Khyber during a one-year survey. The genus *Phlebotomus* was represented by six subgenera and 12 species *viz.* six species of the sub genus *Paraphlebotomus* (*P. alexandri, P. caucasicus, P. kazeruni, P. mongolensis, P. nuri, P. sergenti),* two species of subgenus *Larroussius* (*P. kesheshiani, P. major*) and one species each of the subgenera *Adlerius* (*P. kabulensis), Phlebotomus* (*P. papatasi*), *Euphlebotomus* (specimen damaged, species identification not done) and *Synphlebotomus* (*P. ansari*). *Phlebotomus mongolensis* is reported for the first time from Pakistan.

The genus *Sergentomyia* was represented by 16 species in six subgenera *viz.* subgenus *Parrotomyia* (*S. babu, S. baghdadis, S. bailyi, S. grekovi, S. sumbarica*) and *Sergentomyia* (*S. dentata, S. fallax afghanica, S. mervynae, S. murgabiensis, S. theodori*) were represented by five species each, the subgenera *Grassomyia* (*S. dreyfussi turkestanica, S. indica*), *Sintonius* (*S. cleydei, S. tiberiadis pakistanica*) and *Neophlebotomus* (*S. hodgsoni hodgsoni, S. hodgsoni pawlowskyi*) were having two species each.

Table I. Sand fly species collected from District Khyber with its female to male ratio, relative abundance and resting site preference.

S. No	Species	Number of sand flies			Relative abundance	Status	Sex ratio	% Collected	
		♀	♂	Total				H.D	A.R
1	*P. sergenti*	1710	4028	5738	48.77	D	1:2.4	52.4	47.6
2	*P. papatasi*	661	1444	2105	17.89	D	1:2.2	54.	46
3	*P. alexandri*	165	215	380	3.23	SD	1:1.3	56.6	43.4
4	*P. nuri*	79	121	200	1.70	SD	1:1.5	56	44
5	*P. keshishiani*	34	40	74	0.63	S	1:1.2	52.7	47.3
6	*P. kazeruni*	12	15	27	0.23	S	1:1.25	44.4	55.6
7	*P. caucasicus*	3	8	11	0.09	S	1:2.7	36.4	63.6
8	*P. major*	4	7	11	0.09	S	1:2	63.6	36.4
9	*P. mongolensis*	2	2	4	0.03	S	1:1	100	0
10	*P. ansari*	2	2	4	0.03	S	1:1	100	0
11	*P. kabulensis*	1	2	3	0.02	S	1:2	0	100
12	*S. baghdadis*	765	945	1710	14.53	D	1:1.2	50.4	49.6
13	*S. babu*	360	489	849	7.22	D	1:1.4	48.8	51.2
14	*S. dentata*	25	108	133	1.13	SD	1:4.3	49.6	50.4
15	*S. fx. afghanica*	43	63	106	0.90	S	1:1.5	48.1	51.9
16	*S. clydei*	22	46	68	0.58	S	1:2.1	63.2	36.8
17	*S. dry. turkistanica*	22	39	61	0.52	S	1:1.5	66.7	33.4
18	*S. tib. pakistanica*	25	38	63	0.36	S	1:1.8	54.1	45.9
19	*S. grekovi*	16	26	42	0.53	S	1:1.6	57.1	42.9
20	*S. mervynae*	10	32	42	0.36	S	1:3.2	52.4	47.6
21	*S. ho. hodgsoni*	18	24	42	0.36	S	1:1.3	50	50
22	*S. ho. pawlowskyi*	14	27	41	0.35	S	1:1.9	48.8	51.2
23	*S. murgabiensis*	6	12	18	0.15	S	1:2	61.1	38.9
24	*S. bailyi*	6	12	18	0.15	S	1:2	38.9	61.1
25	*S. theodori*	2	4	6	0.05	S	1:2	66.7	33.3
26	*S. sumbarica*	2	3	5	0.04	S	1:1.5	60	40
27	*S. indica*	2	3	5	0.04	S	1:1.5	100	0
		4011	7755	11766			1:1.93	47.58	52.42

H.D, Human Dwelling; A.R, Animal Room; D, Dominant; S.D, Sub Dominant; S, Satellite; P, *Phlebotomus*; S, *Sergentomyia.*

Relative abundance, sex ratio and resting site preference

Collectively the relative abundance of sand flies in the study area indicated that genus *Phlebotomus* (72.72%) was more abundant as compared to *Sergentomyia* (27.28%). *Phlebotomus sergenti, P. papatasi, S. baghdadis* and *S. babu* were the dominant species in the collection. *P. alexandri, P. nuri* and *S. dentata* were subdominant while remaining species were satellite species according to their abundance (Table I). More males were collected during the survey as compared to females with over all female to male ratio for the collection was 1:1.93. The ratio for different species ranged from 1:1 to 1:4.3 (Table I). Slightly more sand flies (52.42%) were collected from animal rooms as compared to human dwellings (47.58%). Similar observations were reported for majority of the species (Table I). Degree of presence data showed that majority of the species were constant species, two species were frequent, four were moderate, a single species was infrequent while four species were sporadic and collected from a single collection site (Table II).

Table II. Relative abundance of sand flies collected from various sites of District Khyber with its degree of presence (C) during the study period.

S. No	*Species*	Jamrud	Shah Kass	Gudar	Sur Kamar	Ali Masjid	Landi Kotal	Qambar Khel	C	Status
1	*P. sergenti*	920	707	853	1016	940	844	458	100	c
2	*P. papatasi*	287	150	354	386	140	284	504	100	c
3	*P. alexandri*	46	61	47	72	67	58	29	100	c
4	*P. nuri*	28	24	25	42	34	16	31	100	c
5	*P. keshishiani*	7	5	17	20	5	2	18	100	c
6	*P. kazeruni*	4	3	11	7	-	2	-	71.4	f
7	*P. caucasicus*	-	4	7	-	-	-	-	28.6	i
8	*P. major*	1	-	-	3	5	2	-	57.1	m
9	*P. mongolensis*	-	-	-	-	4	-	-	14.3	s
10	*P. ansari*	-	-	-	-	4	-	-	14.3	s
11	*P. kabulensis*	-	-	-	-	3	-	-	14.3	s
12	*S. baghdadis*	180	134	298	436	165	262	235	100	c
13	*S. babu*	46	58	78	159	323	72	113	100	c
14	*S. dentata*	8	6	12	36	4	17	50	100	c
15	*S. fx. afghanica*	4	3	19	29	4	6	41	100	c
16	*S. clydei*	9	2	5	20	17	7	8	100	c
17	*S. dry. turkistanica*	9	8	10	18	9	7	-	85.7	c
18	*S. tib. pakistanica*	7	7	9	16	4	4	16	100	c
19	*S. grekovi*	8	7	7	11	5	4	-	85.7	c
20	*S. mervynae*	4	6	5	10	9	8	-	85.7	c
21	*S. ho. hodgsoni*	3	3	6	17	3	10	-	85.7	c
22	*S. ho. pawlowskyi*	-	-	5	9	8	-	19	57.1	m
23	*S. murgabiensis*	2	2	3	3	7	1	-	85.7	c
24	*S. bailyi*	-	3	2	7	3	3	-	71.4	f
25	*S. theodori*	3	1	-	1	-	1	-	57.1	m
26	*S. sumbarica*	1	-	2	-	2	-	-	42.9	m
27	*S. indica*	-	-	-	-	5	-	-	14.3	s
		1577	1194	1775	2318	1770	1610	1522		

c, constant; f, frequent; m, moderate; i, infrequent; s, sporadic.

Table III. Overall and collection site wise diversity indices for sand fly collected from District Khyber.

S. No	Species	Jamrud	Shah Kass	Gudar	Sur Kamar	Ali Masjid	Landi Kotal	Qambar Khel	Over all
1	Richness	20	20	21	21	24	20	12	27
2	Abundance (%)	13.5	10.1	15.1	19.7	15.0	13.7	12.9	
3	Shannon-Weiner	1.4	1.47	1.62	1.77	1.59	1.49	1.76	1.66
4	Simpson	0.61	0.62	0.7	0.74	0.67	0.66	0.67	0.7
5	Evenness	0.46	0.49	0.53	0.58	0.5	0.5	0.71	0.5

Table IV. Month wise diversity indices of sand flies collected from District Khyber during the study period.

S. No	Species	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
1	Richness	3	15	18	22	24	20	17	15	9
2	Abundance	0.2	8.7	15.8	15.4	23.5	18.6	11	6.3	0.4
3	Shannon-Weiner	0.62	1.56	1.78	1.76	1.69	1.49	1.44	1.13	1.62
4	Simpson	0.64	0.68	0.74	0.73	0.74	0.65	0.65	0.49	0.73
5	Evenness	0.57	0.57	0.61	0.57	0.53	0.5	0.51	0.42	0.73

Diversity indices

Over all and site wise diversity indices including richness, relative abundance, Shannon-Weiner index, Simpson diversity index and evenness were calculated and are shown in Table III. Relative abundance value was highest for Sur Kamar (19.7%), while lowest for Shah Kass (10.1%). Shannon-Weiner index was high for all collection sites which could be explained by the presence of dominant species like *P. sergenti,* as this species alone represent more than 50% of the collection at each site (Table I). Due to high species richness at all collection sites, species evenness values were moderate (0.46 to 0.58) but at Qambar Khel (0.71) it was high, where species richness was lowest.

Seasonal variation in species composition and relative abundance of sand flies

Information regarding seasonal activity and relationship with different climatic variables is given in Figure 2. It has been observed that seasonal activity of sand fly extends from April to December and no sand flies were collected during the cold months of January to March, while only few specimens of sand flies were collected in the months of December and April. High population densities of sand flies were observed in monsoon and post-monsoon months from July to September. Highest densities of sand flies were recorded in the months of August and September (Table IV). Month-wise diversity indices (Table IV) also shows seasonality in occurrence of sand flies. The population of *P. sergenti* peaked in the month of September while that of *P. papatasi* peaked in the month of August. Both these important vector species occur throughout the active season of the study period. Overall, highest density of sand flies was recorded in the month of August. Very little number of specimens belonging to few species were collected in April and in the cold month of December (Table IV). Comparing sand fly abundance with climatic variables have shown that an increase in abundance was observed after rainfall during the hot months when high relative humidity coupled with favourable temperature. Relationship of abundance with climatic variables have shown a very weak and non-significant correlation with humidity and rainfall but a strong positive and significant correlation was observed for temperature ($P<0.05$).

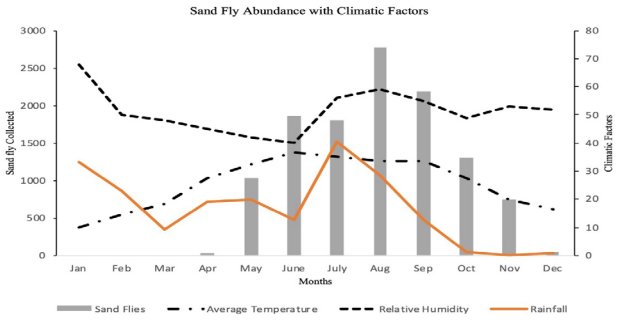

Fig. 2. Sand fly abundance with climatic variables during different months of the year.

DISCUSSION

The ecology of Pakistan supports a variety of insect species including sand flies and mosquitoes and has been endemic for vector borne disease like malaria, dengue, chikungunya and leishmaniasis (Zaidi *et al*., 2017). According to provincial health department of Khyber Pakhtunkhwa more than 40,000 cases of CL have been reported from tribal district Khyber alone during the past

one decade. The present study is the first detailed research work on the ecology of sand flies in different biotopes of rural communities of district Khyber; a major focus of ACL in the northwest of Pakistan. Our study yielded a total of 28 species of sand flies. Eleven species belonging to the genus *Phlebotomus* and sixteen to *Sergentomyia* were collected from indoor-resting sites in seven selected localities of tribal district Khyber. *Phlebotomus. mongolensis* is reported for the first time from Pakistan, thus adding one more species to the sand fly checklist of Pakistan and increasing the number of species in genus *Phlebotomus* to 26 and overall species number to 51.

Species reported in current study had been reported in other studies conducted in the hilly areas of northern Pakistan (Munir, 1994), surveys conducted by Aslamkhan (1998), from Balochistan (Kakarsulemankhel, 2003, 2004), Dir (Ali *et al*., 2016b), North Waziristan (Ali *et al*., 2016a), Bajaur (Wahid *et al*., 2020), Chitral and Nowshera (Rasheed *et al*., 2023). *Phlobotomus. mongolensis* occurrence in Pakistan was predicted by Lewis (1967), but it is now reported for the first time in current study from endemic area of district Khyber. This species inhabits highlands with semi desert like conditions as found in the current study. *Sergentomyia mervynae, S. murgabiensis* and *S. indica* are reported for the first time from the province of Khyber Pakhtunkhwa.

Relative abundance data has shown that sand flies of genus *Phlebtotomus* (72.72%) were more abundant as compared to *Sergentomyia* (27.28%) in the collection done from tribal district Khyber of Pakistan. Similar results have been reported in other studies carried out in leishmaniasis endemic areas of Pakistan (Ali *et al*., 2016a, b; Wahid *et al*., 2020). Among the 27 species reported in present study *Phlebotomus sergenti* (48.77%) was the most abundant species. Overall value of Shannon-Weiner index as well as for each site has been pointing towards the presence of a single species constituting a large proportion of the sand fly population (Al-Koleeby *et al*., 2022) like *P. sergenti* in present study. Studies conducted in Pakistan have also reported similar results. Similar pattern of relative abundance has been reported from the tribal districts of North Waziristan (Ali *et al*., 2016a) and Bajaur in Pakistan (Wahid *et al*., 2020). Contrary to our findings, a study conducted in district Dir reported *P. salengensis,* a mountain dwelling species highest in number followed by *P. sergenti,* while *P. papatasi* was among the least occurring species in the district Dir (Ali *et al*., 2016b). This could explain the endemicity of urban cutaneous leishmaniasis in district Khyber and North Waziristan and sporadicity of the disease in Dir (Rowland *et al*., 1999) where in the later, the vector species (*P. sergenti* and *P. papatasi*) were present in low abundance.

In contrast to the current work, the study conducted in Iran (Hazratian *et al*., 2011; Kassiri and Javedian, 2012; Vatandoost *et al*., 2018; Vahabi *et al*., 2016), Turkey (Kasap *et al*., 2009) and Jordon (Kamhawi *et al*., 1995) have reported *P. papatasi* as the most abundant species as compared to *P. sergenti*. *Phlebotomus papatasi* had been incriminated as proven vector of *L. major* (Maroli *et al*., 2013; Vahabi *et al*., 2016) and in all these studies where *P. papatasi* has been reported to be more abundant were conducted in rural cutaneous leishmaniasis endemic area where *L. major* was the disease-causing agent (El-Sawaf *et al*., 1989; Yaghoobi-Ershadi and Javedian, 1995; Vahabi *et al*., 2016). As current study area is endemic for *L. tropica* (Khan *et al*., 2016), which could explain the abundance of *P. sergenti* as compared to *P. papatasi,* secondly *P. sergenti* is a mountainous species while *P. papatasi* occur in plain areas, which could also explain the abundance of *P. sergenti* or *P. papatasi* on the basis of topography in the above-mentioned studies (Kasap *et al*., 2009; Kassiri and Javedian, 2012).

Among the seven collection sites of the present study no major differences in diversity indices have been observed among these sites. Species richness was observed to be same in all collection sites except Qamber Khel. Qambar Khel is a plain area while remaining sites are foot hills which resulted in differences in the diversity and abundance of sand flies at this collection site. Differences were also present in abundance of sand flies in Qambar Khel and other areas. *Phlebotomus papatasi* is a species of plain area (Kassiri and Javedian, 2012; Vatandoost *et al*., 2018), which explains its abundance in Qambar Khel, while species like *P. sergenti*, *P. alexandri, P. mongolensis, P. major, S. sumbarica* and other species that prefer hilly areas were absent or reported in less abundance from Qambar Khel (Kassiri and Javedian, 2012). Qambar Khel was also having a more urban set up with less animal sheds for keeping cattle, goat and sheep which along with urbanization, topography and habitat scarcity could explain low diversity of sand flies at this site. Remaining six sites were having almost the same habitat and differences in diversity of species exist only for those that were less abundant in the collection (Table I).

Two types of indoor resting sites i.e., animal rooms and human dwellings were used for collection of sand flies. More sand flies were collected from animal rooms (52.42%) than human dwellings (47.58%). Though this difference was small, but could be attributed to less disturbance, more resting sites and construction type of animal rooms (mostly mud walls and thatched roofs), that can provide better resting sites to these flies during daytime. Other reasons may include increase in human population which has changed host preferences from wild animals to human and domesticated animals and shift in breeding places from rodent's burrows and wild animal

resting sites to livestock ranches, barns and animal rooms in domestic dwellings. Presence of cattle and domestic animals in previously uninhabited areas not only replace rodents and wild animals as food but has also shifted breeding places of sand flies to cattle sheds (Vatandoost *et al*., 2018).

Sex ratio of sand flies tends to remain 1:1 in nature (Killick-Kendrick, 1999) but our study has reported more males as compared to females. The overall female to male ratio was 1:1.9 for the study period in district Khyber. Important vector species like *P. sergenti* and *P. papatasi* have sex ratio of 1: 2.4 and 1: 2.2, respectively. Similar observations had been reported by other studies conducted in Pakistan where more males were collected as compared to females (Ali *et al*., 2016a, b; Wahid *et al*., 2020). Presence of higher frequency of males as compared to females could be attributed to various factors like presence of indoor larval habitats (Vatandoost *et al*., 2018), availability of more host for blood feeding to females, which attract males also as males arrive first at the feeding site seeking for a female to mate with (Dye *et al*., 1991). Studies had shown that at higher densities of sand flies the sex ratio is more male biased (Quinnell and Dye, 1994). In our study area, people keep their animals indoor during the daytime to protect them from the heat of hot summers and outdoor at night. As current study used flit method for collection of sand flies from their indoor resting sites in human dwellings and animal rooms after sun rise and before sunset, female were taking shelters in these rooms followed by males which could be the probable reason for male biased sex ratio (Kasap *et al*., 2009).

Knowledge regarding distribution, abundance and seasonal variation help in deciding the correct time and strategy to implement control programs in an area (Galvez *et al*., 2010). Present study shows a single peak of abundance during the month of September and occurrence only during the warm months and relatively cold months of November and December. Seasonal activity of sand flies is greatly influenced by climatic variables like temperature, relative humidity and rainfall (Maroli *et al*., 2013). In district Khyber there are two spells of rain, one is winter and the other is summer. Winter spell occur in the month of January and summer during the monsoon period from July to September, however, a positive effect of temperature along with relative humidity on sand fly population has been observed during the monsoon rains of summer. A positive correlation of temperature with population abundance has been reported for sand flies (Gebresilassie *et al*., 2015). An increase in abundance of overall sand flies has been observed during these months with population density reaching its maximum in August, when temperature and relative humidity both were comparatively high as compared to other months. Similar results have been reported in other parts of the country where single peak of sand flies was observed during the active season. In North Waziristan the active season has been reported to be shorter than present study from April till September with a peak in the month of July (Ali *et al*., 2016a) and in Dir also the active season ranged from May to October with a peak in July and August (Ali *et al*., 2016b). In district Bajaur unimodal distribution with active season ranging from June to October has been reported by Wahid *et al*. (2020). Temperature and specifically relative humidity had been observed to play an important role in the seasonal activity of sand flies in such areas (Srinivasan *et al*., 2013).

It has been reported that in colder climatic zones sand flies have shorter active season (Hazratian *et al*., 2011; Ali *et al*., 2016a, b), while area with warm temperate climate have lengthy active season (Mirhoseni *et al*., 2017). Studies have shown that in arid and semi-arid biotopes sand flies have short active season with bimodal distribution while warm temperate areas have lengthy active season with unimodal distribution (Hazratian *et al*., 2011). District Khyber lies in temperate warm zone, which explains lengthy active season with single density peak immediately after rainy season due to high relative humidity coupled with high temperature resulting in increase in abundance of sand flies during these months.

CONCLUSION

Among the reported species *P. papatasi, P. sergenti, P. alexandri, P. salehi, P. keshishiani* and *P. major* (Kassiri and Javedian, 2000) had been proven as vectors of various *Leishmania* parasites in other parts of the world. This shows that our study area is at risk of an increase in disease incidence and spread of *Leishmania major* and visceral leishmaniasis. In conclusion, the abundance of *P. sergenti* as compared to other sand fly species such as *P. patasi,* the geographical conditions and circulation of *L. tropica* in the local population explain the endemicity of ACL or urban cutaneous leishmaniasis in district Khyber. The low abundance of sand fly species of subgenera *Larusius* and *Adlerius* in this geographical region shows the possibility of sporadic VL cases in the region. More epidemiological studies are required along with continuous vector surveillance in the field of cutaneous as well as visceral leishmaniasis to decrease the disease incidence of CL and prevent cases of VL in the area.

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IRB approval and ethical approval

This study was reviewed, approved and monitored by the Ethical Committee, Faculty of Life and Environmental Sciences, University of Peshawar, under applicant certificate number (12/EC/F.LIFE-2020).

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Ali, N., Ullah, A., Wahid, S., Khisroon, M., and Rasheed, S.B., 2016a. Distribution, species composition and relative abundances of sand flies in North Waziristan Agency, Pakistan. *Med. Vet. Ent.,* **30**: 89-94. https://doi.org/10.1111/mve.12141

Ali, N., Khan, K., Wahid, S., Khan, N.H. and Shah, S.U., 2016b. Species composition and activity patterns of sand flies (Psycodidae: Phlebotomine) in four tehsils of Dir Districts, Khyber Pakhtunkhwa, Pakistan. *Acta Trop.*, **156**: 144-151. https://doi.org/10.1016/j.actatropica.2016.01.011

Al-Koleeby, Z., Aboudi, A.E., Bortel, W.V., Cloots, K., Benkirane, R., Faraj, C. and Talbi, F.Z., 2022. Ecological survey of the peridomestic sand flies of an endemic focus of zoonotic cutaneous leishmaniasis in the south-east of Morocco. *Sci. World J.,* **2022**: 5098005. https://doi.org/10.1155/2022/5098005

Artemiev, M.M., 1976. *Sandflies (Diptera, Psychodidae, Phlebotominae) of Afghanistan*. Marzinovoskyi Institute of Medical Parasitology and Tropical Medicine, Moscow.

Ashford, R.W., 1996. Leishmaniasis reservoirs and their significance in control. *Clin. Dermatol.*, **14**: 523-532. https://doi.org/10.1016/0738-081X(96)00041-7

Aslamkhan, K., Aslamkhan, M. and Azizullah. 1998. Biodiversity of sand flies of Pakistan and Kashmir. *Pakistan J. Zool.*, **30**: 13-21. https://doi.org/10.4269/ajtmh.1972.21.30

Dye, C., Davies, C.R. and Lainson, R., 1991. Comunication among phlebotomine sandflies: A field study of domesticated *Lutzomyia longipalpis* populations in Amazonian Brazil. *Anim. Behav.,* **42**: 183-192. https://doi.org/10.1016/S0003-3472(05)80549-4

El-Sawaf, B.M., Mansour, N.S.; El-Said, S.M., Daba, S., Youssef, F.G., Kenawy, M.A. and Beier, J.C., 1989. Feeding patterns of *Phlebotomus papatasi* and *Phlebotomus langeroni* (Diptera: Psychodidae) in el agamy, Egypt. *J. med. Ent.*, **26**: 497-498. https://doi.org/10.1093/jmedent/26.5.497

Galvez, R., Descalzo, M.A., Micro, G., Jimenez, M.I., Martin, O. and Dos Santos-Brandao, F., Guerrero, I., Cubero, E. and Molina, R., 2010. Seasonal trends and spatial relations between environmental/meteorological factors and leishmaniasis sand fly vector abundances in central Spain. *Acta Trop.*, **115**: 95-102. https://doi.org/10.1016/j.actatropica.2010.02.009

Gebresilassie, A., Abbasi, I., Aklilu, E., Yared, S., Kirstein, O.D., Moncaz, A. and Gebre-Michael, T., 2015. Host-feeding preference of *Phlebotomus orientalis* (Diptera: Psychodidae) in an endemic focus of visceral leishmaniasis in northern Ethiopia. *Parasites Vectors,* **8**: 1-8. https://doi.org/10.1186/s13071-015-0883-5

Hazratian, T., Vatandoost, H., Oshaghi, M.A., Yaghoobi-Ershadi, M.R., Fallah, E., Rafizadeh, S. and Rassi, Y., 2011. Diversity of sand flies (Diptera: Psychodidae) in endemic focus of visceral leishmaniasis in Azar Shahr District, East Azarbaijan Province, north west of Iran. *J. Arthrop. Borne Dis.,* **10**: 328.

Kakarsulemankhel, J.K., 2004. Checklist and distributional records of Phlebtomine Sand flies from Balochistan Province, Pakistan (Diptera, Psychodidae). *Pakistan J. biol. Sci.,* **7**: 593-596. https://doi.org/10.3923/pjbs.2004.593.596

Kakarsulemankhel, J.K., 2003. Snad fly (Diptera, Psychodidae, Phlebotominae) fauna of South-Western Pakistan. 1. Diagnostic morphology of *Phlebotomus papatasi* (Scopoli), *P. bergeroti* (Parrot) and *P. salehi* (Meshhali). *Parassitologia,* **45**: 103-118.

Kamhawi, S., Abdel-Hafez, S.K. and Molyneux, D.H., 1995. A comprehensive account of species composition, distribution and ecology of phlebotomine sandflies in Jordon. *Parasite*, **2**: 163-172. https://doi.org/10.1051/parasite/1995022163

Kasap, O.E., Belan, A., Kaynas, S., Simsek, F.M., Biler, L., Ata, N. and Alten, B., 2009. Activity patterns of sand fly (Diptera: Psychodidae) species and comparative performance of different traps in an endemic cutaneous leishmaniasis focus in Cukurova plain, Southern Anatolia, Turkey. *Acta Vet. Brno*, **78**: 327-335. https://doi.org/10.2754/avb200978020327

Kassiri, H. and Javadian, E., 2012. Composition of the sand fly fauna in Khash County, Southeast Iran. *J. Insect Sci.*, **12**: 132. https://doi.

org/10.1673/031.012.13201

Kassiri, H., Javadian, E. and Seyedi-Rashti, M.A., 2000. List of phlebotominae (Diptera: Psychodidae) of Iran. *Bull. Soc. Pathol. Exot.*, **93**: 129-130.

Khan, N.H., Bari, A.U., Hashim, R., Khan, I., Muneer, A., Shah, A., Wahid, S., Yardley, V., O'Neil, B. and Sutherland, C.J., 2016. Cutaneous leishmaniasis in Khyber Pakhtunkhwa Province of Pakistan: Clinical diversity and species-level diagnosis. *Am. J. trop. Med. Hyg.*, **95**: 1106-1114. https://doi.org/10.4269/ajtmh.16-0343

Killick-Kendrick, R., 1999. The biology and control of phlebotomine sand flies. *Clin. Dermatol.*, **17**: 279-289. https://doi.org/10.1016/S0738-081X(99)00046-2

Lane, R.P. and Crosskey, R.W., 1993. *Medical insects and arachanids.* Chapman and Hall, London. https://doi.org/10.1007/978-94-011-1554-4

Lewis, D.J., 1978. The phlebotomine sandflies (Diptera: Psychodidae) of the Oriental region. *Bull. Br. Mus. Nat. Hist. Zool.*, **37**: 217-343.

Lewis, D.J., 1967. The phlebotomine sand-flies of West Pakistan (Díptera: Psychodidae). *Bull. Br. Mus. Nat. Hist. Ent.,* **19**: 3-57. https://doi.org/10.5962/bhl.part.28298

Lewis, D.J., 1982. A taxonomic review of the genus *Phlebotomus* (Diptera: Psychodidae). *Bull. Br. Mus. (Nat. Hist.) Ent. Ser.,* **345**: 121-209.

Maroli, M., Feliciangeli, M.D., Bichaud, L., Charrel, R.N. and Gradoni, L., 2013. Phlebotomine sand flies and the spreading of leishmaniasis and other diseases of public health concern. *Med. Vet. Ent.*, **27**: 123-147. https://doi.org/10.1111/j.1365-2915.2012.01034.x

Mirhoseini, M., Salehzadeh, A., Ramazan-Jamaat, S., Zahirnia, A.H. and Rahmanzadeh, N., 2017. Distribution and seasonal activity of phlebotominae sand flies in Yazd and its outskirts, center of Iran. *Sci. World J.,* **2017**: 1486845. https://doi.org/10.1155/2017/1486845

Munir, M.A., 1994. *Systematics and biology of Phlebotomine sandflies of the Visceral leishmaniasis foci of Northern Pakistan*. PhD thesis. School of Hygiene and Tropical Medicine, London.

Quinnell, R.J. and Dye, C., 1994. Correlates of the peridomestic abundance of *Lutzomyia longipalpis* (Diptera: Psychodidae) in Amazonian Brazil. *Med. Vet. Ent.*, **8**: 219-224. https://doi.org/10.1111/j.1365-2915.1994.tb00502.x

Qutubuddin, M., 1951. The sand fly fauna of Kohat Valley, NWFP. *Pakistan J. Hlth.*, **1**: 34–36

Rasheed, S.B., Zaidi, F., Samiullah and Jan, T., 2023. Sand Fly (Psychodidae: Phlebotominae) ecology of an emerging cutaneous leishmaniasis focus in Khyber Pakhtunkhwa with an Updated Species Checklist from Pakistan. *Pakistan J. Zool.,* (In press). https://doi.org/10.17582/journal.pjz/20221219171215

Rowland, M., Munir, A., Durrani, N., Noyes, H. and Reyburn, H., 1999. An outbreak of cutaneous leishmaniasis in an Afghan refugee settlement in north-west Pakistan. *Trans. R. Soc. trop. Med. Hyg.*, **93**: 133-136. https://doi.org/10.1016/S0035-9203(99)90285-7

Srinivasan, R., Jambulingam, P. and Vanamail, P., 2013. Sand fly (Diptera: Psychodidae) abundance andspecies diversity in relation to environmental factors in parts of coastal plains of southern India. *J. med. Ent.,* **50**: 758-763. https://doi.org/10.1603/ME12153

Vahabi, A., Rassi, Y., Oshaghi, M.A., Sayyadi, M. and Rafizadeh, S., 2016. Detection of Leishmania major DNA within wild caught *Phlebotomus papatasi* and species composition of sand flies in endemic focus of cutaneous leishmaniasis, in Western Iran. *J. Parasit. Dis.,* **40**: 69-74. https://doi.org/10.1007/s12639-014-0448-0

Vatandoost, H., Nejati, J., Saghafipour, A. and Zahraei-Ramazani, A., 2018. Geographic and ecological features of phlebotomine sand flies (Diptera: Psychodidae) as leishmaniasis in Central Iran. *J. Parasit. Dis.,* **42**: 43-49. https://doi.org/10.1007/s12639-017-0962-y

Wahid, S., Khan, K. and Khan, N.H., 2020. Sand fly (Diptera: Psychodidae) species diversity, habitat preferences, and ecological aspects of distribution in Bajaur District, Khyber Pakhtunkhwa, Pakistan. *J. med. Ent.,* **57**: 1432-1439. https://doi.org/10.1093/jme/tjaa050

Wessa P., 2017. *Pearson correlation (v1.0.13) in free statistics software* (v1.2.1). Office for Research Development and Education. https://www.wessa.net/rwasp_correlation.wasp/

Yaghoobi-Ershadi, M.R. and Javadian, E., 1995. Zoonotic cutaneous leishmaniasis to the north of Isfahan. Human infection in 1991. *Bull. Soc. Pathol. Exot.,* **88**: 42-45.

Zaidi, F., Fatima, S.H., Jan, T., Fatima, M., Ali, A., Khisroon, M. and Rasheed, S.B., 2017. Environmental risk modeling and potential sand fly vectors of cutaneous leishmaniasis in Chitral district: A leishmanial focal point of mount Tirich Mir, Pakistan. *Trop. Med. Int. Hlth.,* **22**: 1130-1140. https://doi.org/10.1111/tmi.12916