"Kaal Daana" (Cutaneous Leishmaniasis) in South-West Pakistan: A Preliminary Study

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SUMMARY: The incidence and prevalence of "Kaal Daana" (cutaneous leishmaniasis), sand fly species and rodent reservoirs were studied in seven geographical areas in south-western Pakistan (Balochistan). Fifteen thousand eight hundred and forty seven persons (5-40+ years in age) were examined. Eight thousand and seven (50.52 %) had active lesions and 7529 (47.51 %) had scars resulting from past infection with CL. A total of 2013 sand flies were collected indoors and from rodent burrows using an aspirator and more often with sticky traps. Seven species of sand flies of the genus *Phlebotomus* and 16 species of the genus *Sergentomyia* were identified. Forty-one of the one hundred thirty nine rodents trapped alive belonged to the *Meriones* sp. and the remaining included 61 *Rattus rattus*, twenty-one *Tatera indica* and 16 *Mus musculus*. One *Meriones* sp. was positive for amastigotes in smears prepared from the ears of the animal.

Key words: "Kaal Daana", cutaneous leishmaniasis (CL), sand flies, south-western Pakistan

Pakistan'ın Güneybatısında Deri Leishmaniasisi: Ön Çalışma

ÖZET: Deri leishmaniasisinin insidansı ve prevalansı, kum sineklerinin türleri ve kemirici rezervuarlar güneybatı Pakistan'daki (Balochistan) 7 coğrafik bölgede çalışılmıştır. 15.847 kişi (5-40+ yaş arası) incelenmiştir. 8007 (%50.52) kişide aktif lezyon saptanırken, 7529 (%47.561) kişide daha önce geçirilen deri leishmaniasisinden kalan scar tesbit edilmiştir. Toplam 2013 kum sineği örneği ev içlerinden, kemirici yuvalarından ağız aspiratörü ve yağlı kağıt tuzaklar kullanılmak suretiyle yakalanmıştır. *Phlebotomus* genusuna ait 7, *Sergentomyia* genusuna ait 16 tür tanımlanmıştır. Canlı olarak yakalanan 139 kemiricinin 41'inin *Meriones* türleri, 61'inin *Rattus rattus*, 21'inin *Tatera indica* ve 16'sının *Mus musculus* olduğu görülmüştür. Hayvanların kulaklarından yapılan yayma preparatlarda incelenmiş ve sadece bir *Meriones* örneğinde amastigotlar görülmüştür.

Anahtar kelimeler: Deri leishmaniaisi, kum sinekleri, güneybatı Pakistan

GİRİŞ

The disease cutaneous leishmaniasis (CL) here is called by its famous local name "Kaal Daana", meaning in "Pashto", a sore taking at least one year to heal. Both the CL as well as the visceral form of the disease is reported from various parts of the country. While CL cases have been recorded in the most northern part of the country in the Gilgit area, the majority of cases occur in SW Pakistan (Balochistan) (1,6,18,17,4,7). CL which was reported to be prevalent in only a couple of Districts have now spread to the entire province (9). Reviews on leishmaniasis have also been published by Haq (5), Munir *et al.* (15), Masoom and Marri (14), WHO (23) and Yasinzai *et al.* (25). Balochistan is in fact the biggest province of Pakistan with a vast area having an extreme and varied hot and cold climate. It is situated at the cross road of Afghanistan, Iran and India and shows the highest incidence of CL in the country.

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Although CL is reported from various parts of the country such as northern areas, NWFP, Multan and Karachi (13,16), the incidence of CL in SW Pakistan is extensive. Prevalence appear to be much high in Persian, Pashton, Tajak, Uzbek and Turkmen tribes migrated from Afghanistan and Iran. Both Anthroponotic cutaneous leishmaniasis (Urban or Dry type) as well as Zoonotic cutaneous leishmaniasis (Rural or wet type) occurs in the region although the latter is more prevalent (19,2). The disease manifests itself mainly in children and non-immune adults. It was aimed in the present study to determine the age, sex, location distribution of the cases of CL mainly among school children and peoples of higher age in seven geographical zones in SW Pakistan and to identify the sand flies found in the region and possible rodent reservoir (s) of the disease.

MATERIALS AND METHODS

A survey of the disease was carried out by the present author in endemic areas in SW Pakistan (Balochistan) during 1996-2001. This includes data collected from various District Hospitals, private clinics and school children and boys of

endemic areas of seven zones of the Province (Table 1, 2 and 3). The endemic areas have been divided into seven zones keeping in view of their geographic and climatic homogeneity to some extent. The techniques discussed by Rab *et al*. (18) were generally followed.

Population studies: Balochistan (SW Pakistan) is in fact the biggest province of Pakistan with a vast area having an extreme and varied hot and cold climate. It is situated at the cross road of Afghanistan, Iran and India and shows the highest incidence of CL in the country. It has a 347,190 area sq. km. (43.61%) (Pakistan, 796, 096 area sq. km.), 19 person density per person sq. km. (Pakistan,164 person density per sq. km.) and a population over 7 million (Pakistan, a population over 130 million) with an annual rate of increase of about 5.3% (Source: Census :1998, Pakistan Statistical Year Book 2000, published by Federal Bureau of Statistics, Statistical Division, Govt. of Pakistan, p.14). The location surveyed include the High, Middle and Primary school children (boys and girls), hospitals and private clinics. School children, boys and girls (of 5 to 16 years) and persons of higher age including teachers, Government servants and laborers (aged up to 40 years) were examined for active lesions and scars resulting from the disease. Careful history regarding the development of scars was obtained and those due to conditions other than Leishmaniasis such as trauma, burns, etc. were excluded. The diagnosis of the disease was done mainly on clinical and epidemiological criteria and by microscopic examination of amastigotes in scrapping from ulcer margins after fixing and staining with leishman stain. Data of infection of CL was collected from various hospitals and private clinics of the province.

Collection of sand flies: Sand flies were collected with the help of transparent plastic sheets (12" x 12 inches) coated with castor oil and also by mouth aspirator. Sand flies were placed in Berlese's medium. Identification was carried out and blood fed or gravid females were dissected for evidence of promastigotes using dissecting and a light microscope.

Collection and examination of rodents: Rodents were caught in locally made traps with spring-operated doors. They were placed amidst active colonies of rodent burrows. Pieces of cheese, carrot, and bread smeared with butter were used as bait. The animals caught were examined for sores and scars. Giemsa touched preparations from ears and tail regions were examined.

RESULTS

The present investigation revealed that incidence of disease in the patients attending hospitals and private clinics in SW Pakistan is around from 25% to 35% of all cases. A total of 15847 persons were recorded, eight thousand seven were found with active lesions, 7529 with scars resulting from leishmania ulcer and three hundred eleven with no symptoms (Table 1 and 2). The ratio of incidence in male/ female is 72**:**28%, may be mainly because the women mostly stay at home. Largest number of patients were surveyed in Schools Children besides in hospitals and private health centers of Quetta, Bela, Khuzdar, Kohlu, Uthal,Turbat, Panjgour, Duki and Chaman locality. The Afghan refugee camps are also situated at a distance of 3-40 miles from Quetta and 5-15 miles from Chaman, therefore, the cases observed in Quetta and Chaman incluede Afghan refugees. Dry lesions were mostly seen in north zone with Quetta city, which is in the north of the SW Pakistan (Balochistan), the area highly exposed to refugees and travelers from Afghanistan and Iran. In the other six regions, mostly wet lesions were seen twice or more than the dry lesions. School survey revealed that a total of 5853, out of 13093 school children examined, had active lesions (44.70%), 6929 had scars resulting from Leishmania ulcer (52.92%), three hundred eleven were found with no symptoms (2.37%) while 3210 with active lesions were from the younger age group of 5-10 years (Table 3).

An epidemic of CL was observed in 1997 at the Jhaljhao locality amongst the non-locals workers of Bela Awaran Road. The majority had multiple lesions of the wet type. The highest incidence of disease is during the winter and early spring. Diagnosis of the disease by microscopic examination of amastigotes in direct smear preparation did not reveal encouraging results, as many of the cases reported negative by the laboratory turned out to be positive when the dermatologists treated the patient with antimonials and the patient was cured.

Zone locality wise distribution and composition of sand flies showing species and their percentages are given in the Table 4, 5 and Map 1. A total of 2013 sand flies (Table 5) were collected from indoor and out door (rodent borrows). Of these,1052 belonged to the *Phlebotomus* genus and the remaining 956 were of the *Sergentomyia* genus. Five sand flies were observed probably to be the new species belonging to the subgenera *Phlebotomus* and *Parratomyia* are not counted here in the number of species as more specimens are required. Seven hundred twenty were identified as *Ph. papatasi* (35.76%), 30 as *Ph. bergeroti* (1.49%), 70 as *Ph. salehi* (3.47%), 140 as *Ph. sergenti* (6.95%), 82 as *Ph. alexandri* (4.07%), 09 as *Ph. nuri* (0.44) and one (male) as *Ph. andrejevi.* Remaining sixteen species were of *Sergentomyia* group (Table 5).

Zone-wise distribution of the species of sand flies are given in the Map of Balochistan Province (Figure 1). 632 gorged and gravid female sand flies were dissected for detection of leptomonad which all proved to be negative. The seasonal activity of most phlebotomine species is bimodal but their relative abundance is different from one area to another. Furthermore, the same species can be either endophilic or exophilic. All the incriminated and suspected vector species in

Table 1. Incidence of "Kaal Daana" (CL) in Balochistan Province according to age

	5-10 yrs	11-16 yrs	17-22 yrs	23-30 yrs	31-40 yrs	Total
Active lesion	3210	2643	1617	433	104	8007
Scar	3823	3106	600	-	-	7529
No symptoms	203	108	-	-	-	311
Total	7236	5857	2217	433	104	15847

Table 2. Incidence of "Kaal Daana" (CL) in Balochistan Province, zoogeographic zone wise showing sex and lesion type

Zones	Main Location	Lesion type						Subtotal
		Male			Female			
		Dry	Wet	Scar	Dry	Wet	Scar	
1. Northwest zone	Chaman, QillaAbdullah, JangalPirAlizai, Pishin, Quetta, Ziarat, Zhob	797	121	-	356	51	-	1325
2. Northeast zone	Loralai, Duki, Nanasahebziarat, Kohlu, Kahan, Rakni, Barkhan, DeraBugti, Sangsilla, Mewand, Musakhel	100	430	-	51	205	-	786
3. Northcentral zone	Dhadar, Sibi, Bhag, Chilgiri, Belpat, Lehri, DeraMuradjamali, DeraAllahYar, Hairdin, Adampur, Sohbatpur, UstaMuhammad	239	1022	-	95	472	-	1828
4. Central south zone	Kalat, Khuzdar, Wadh, Ornach, Nal, Mashkey, Awaran, Jhaljhau	73	310	-	48	160	-	591
5. Uppercentral west zone	Chagi, Noshki, Dalbandin, Kharan, Panjgour, Parom	155	630	-	70	280	-	1135
6. Extreme southeast zone	Bela, Uthal, Winder, Somiani	72	301	-	50	182	-	605
7. Extreme southwest zone	Turbat, Buleda, Tump, Mand, Dashtekuddan, Suntsar, Naillant	228	1007	-	92	410	-	1737
	TOTAL	1664	3821	5706	762	1760	1823	15536

Table 3. Incidence of "Kaal Daana" (CL) in children (5-10 and 11-16 years) in Balochistan Province

Age group	Total number	Active lesion (%)	Scar (%)	No symptom (%)
5-10	7236	3210 (44.36)	3823 (52.83)	203 (2.80)
11-16	5857	2643 (45.12)	3106 (53.03)	108 (1.84)
Total	13093	5853	6929	311

SW Pakistan are present and correlate with the recorded CL cases. The number of porous sand flies during June and July in colder areas and in March and April in warmer areas was greater than other months.

Live traps were placed at two hundred thirty two places and 139 rodents were collected. Except for one *Meriones* sp. from Duki area which showed parasites in smears prepared from the edges of its healthy ears while all the rodents were free from clinical infection (see Table 6). *M. hurrianae* in the Uthal area of SW Pakistan has been found naturally infected with parasites previously (17).

Table 6. Results of screenings of rodents

Species	Number collected	Parasitologically positive
***Meriones* sp.**	41	1
Rattus rattus	61	0
Mus musculus	16	0
Tatera indica	21	0
Total	139	1

Table 4. Zon-Locality-wise distribution of species of Phlebotomine sand flies in SW Pakistan (Balochistan), 1996-2001.

1. North west	Chaman	*Phlebotomus papatasi, P. sergenti, P. salehi, Sergentomyia fallax, S. babu babu, S. baghadadis*
	Qilla Abdullah, PirAlizai; Pishin; Zhob, Ziarat	*P. papatasi, P. sergenti*
	Quetta	*P. papatasi, P. sergenti, P. nuri*
2. North east	Duki	*P. papatasi, S. t. Pashtunica, S. fallax, S. baghdadis, Grassomyia indica, G. d. turkestanica*
	Nanasaheb Ziarat	*P. papatasi, P. salehi, S. murghabiensis, S. t. pashtunica, S. fallax, S. mervynae, S. babu babu, G. indica, G. d. turkestanica, S. hodgsoni hodgsoni*
	Kohlu	*P. papaasi, P. sergenti, P. salehi, S. fallax, S. babu babu, S. baghdadis, S. clydei, S.g. indica, S.g. d. turkestanica*
	Kahan	*P. papatasi, S. murghabiensis, S. fallax, S. babu babu, S. baghdadis, S. clydei, S. g. indica, S. g. d. turkestanica, S. hodgsoni hodgsoni*
	Mewand	*P. papatasi*
	Barkhan	*P. papatasi, S. babu babu*
	Rakhni	*P. papatasi*
	DeraBugti, Sangsilla	*P. papatasi, S. clydei, S. punjabiensis.*
3. Low land	Dhadar	*P. andrejvi, S. t. pashtunica, S. punjabiensis, S. babu babu, S. baghdadis, S. clydei, S.g. indica, S.g. d. turkestanica*
	Sibi	*P. papatasi, P. alexandri, S. t. pashtunica, S. punjabiensis, S. fallax, S. grekovi, S. babu babu, S. baghdadis, S. p. freetownensis, Sinton var., S. clydei, S.g. d. turkestanica.*
	Bhag, Chilgiri, DeraMuradJamali	*P. papatasi*
	Belpat, Lehri	*P. papatasi, S. punjabiensis*
	DeraAllahYar	*P. papatasi, S. babu babu, S. punjabiensis, S.g. indica, S.g. d. turkestanica*
	Adampur, Hairdin	*P. papatasi, S. babu babu, S. punjabiensis, S.g. indica, S.g. d. turkestanica*
	Manjhipur, Sohbatpur	*P. papatasi*
4. Central south	Khuzdar	*P. papatasi, P. salehi, P. bergeroti, P. alexandri, P. nuri, S. murghabiensis, S. t. pashtunica, S. fallax, S. palestinensis, S. babu babu, S. baghdadis, S. p. freetownensis Sinton var., S. clydei, S. t. pakistanica, S.g. indica, S.g. d. turkestanica*
	Wadh	*P. papatasi, P. alexandri, S. babu babu, S. baghdadis*
	Ornach	*P. papatasi, P. sergenti*
	Nal	*P. papatasi, P. alexandri, S. babu babu, S. baghdadis, S. clydei*
	Awaran, JhalJhao, Mashkey	*P. papatasi*
5. Extreme south	Bela	*P. papatasi, P. salehi, P. bergeroti, S. murghabiensis, S. t. pashtunica, S. fallax, S. palestinensis, S. baghdadis, S. p. freetownensis Sinton var., S. clydei, S. t. pashtunica, S.g. indica, S.g. d. turkestanica, S. hodgsoni hodgsoni.*
	Uthal	*P. bergeroti, S. punjabiensis, S. palestinensis, S. p. freetownensis Sinton var*
	Winder	*P. papatasi, S. p. freetownensis Sinton var.*
	Somiani	*P. papatasi*
6. Upper west central	Chagi, Dalbandin, Noshki, Kharan	*P. papatasi*
	Panjgour	*P. papatasi, P. sergenti, P. bergeroti, P. salehi, S. t. pashtunica, S. mervynae, S. babu babu, S. baghdadis, S. clydei*
	Parom	*P. papatasi, S. t. pashtunica, S. clydei, S.g. d. turkestanica*
7. Extreme south west	Turbat	*P. papatasi, P. bergeroti, P. salehi, P. alexandri, S. murghabiensis, S. mervynae, S. fallax, S. baghdadis, S. clydei, S. t. pakistanica, S.g. d. turkestanica*
	Mand	*P. papatasi, S. dentate arpaklensis Perfiliev, S. clydei*
	DashteKuddan	*P. papatasi, P. salehi, S. fallax, S. babu babu, S. baghdadis, S. clydei.*
	Buleda, Naillaint, Suntsar	*P. papatasi*
	Tump	*P. papatasi, S. murghabiensis, S. t. pakistanica, S. fallax, S. clydei.*

Table 5. Composition of sand fly species in endemic areas of cutaneous leishmaniasis in Balochistan, Pakistan

Species		**Suburban**	**Rural**	**Field**
Ph. papatasi	(N=720, 35.76%)	290 (40.2%)	360 (50%)	70 (9.7%)
Ph. bergeroti	(N=30, 1.5%)	15 (50%)	12 (40%)	3 (30%)
Ph. salehi	(N= 70, 3.48%)	22 (31.42%)	28 (40%)	10 (14.28%)
Ph. sergenti	(N=140, 6.94%)	48 (34.28%)	74 (52.85%)	18 (12.85%)
Ph. alexandri	(N=82, 4.07%)	13 (15.85%)	61 (74.39%)	8 (9.75%)
Ph. andrejevi	-	-	a single male	-
Ph. nuri	(N=09, 0.45%)	6 (66.66%)	3 (33.33%)	-
S. fallax	(N=80, 3.97%)	6 (7.5%)	72 (90%)	2 (2.5%)
S. punjabiensis	(N=22, 1.09%)	10 (45.45%)	12 (54.55%)	-
S. murghabiensis	(N=17, 0.84%)	-	17 (100%)	-
S. mervynae	(N=19, 0.94%)	-	19 (100%)	-
S. dentate arpaklensis	(N=2, 0.1%)	-	02 (100 %)	-
S. theodori pashtunica	(N=158, 7.85%)	-	154 (97.6%)	4 (2.53%)
S. babu babu	(N=165, 8.2%)	40 (24.24%)	125 (75.76%)	-
S. palestinensis	(N=10, 0.5%)	10 (100%)	-	-
S. baghdadis	(N=145, 7.2%)	55 (37.93%)	90 (62.07%)	-
S. grekovi	(N=1)	a single female	-	-
S. freetownensis var	(N=110,5.46%)	47 (42.72%)	58 (52.73%)	5 (4.55%)
S. clydei	(N=75, 3.73%)	22 (29.33%)	53 (70.67%)	-
S. tiberiadis pakistanica	(N=25, 1.24%)	15 (60%)	10 (40%)	-
S. hodgsoni hodgsoni	(N=20, 1%)	4 (20%)	16 (80%)	-
S. g. indica	(N=54, 2.68%)	17 (31.48%)	37 (68.52)	-
S. g. dreyfussi turkestanica	(N=53,2.63%)	21 (39.62%)	32 (60.38%)	-

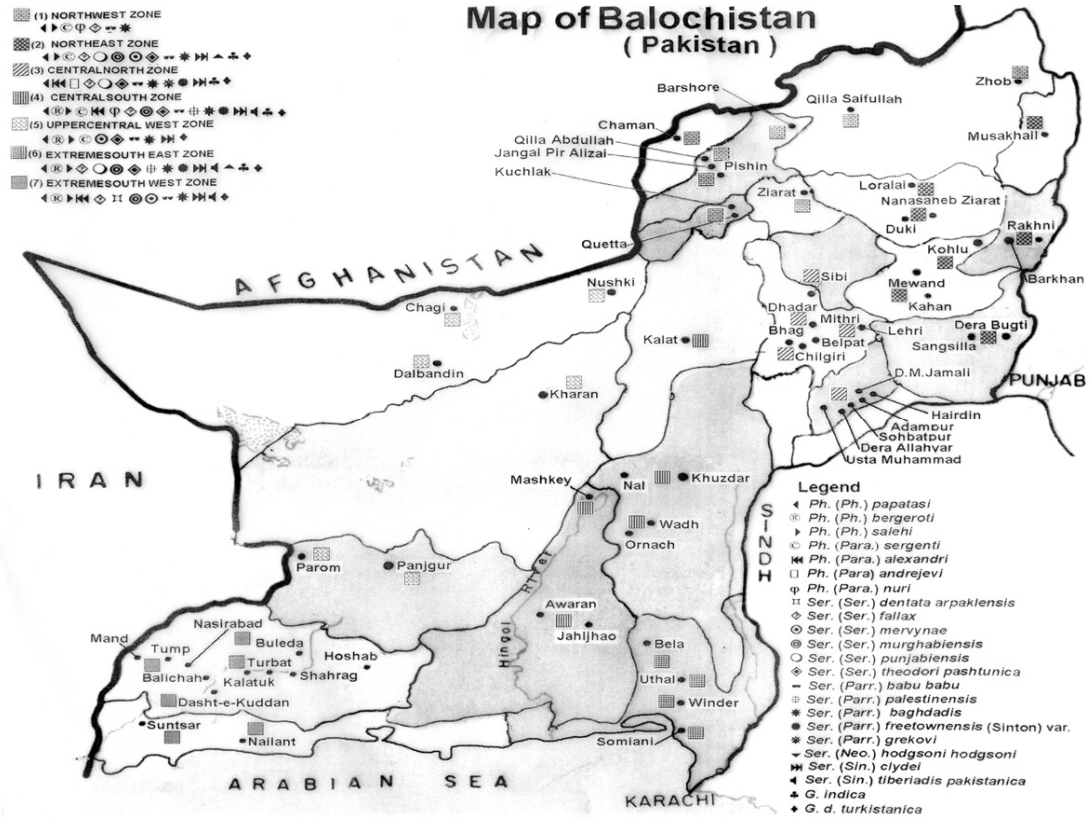

Figure 1. Distribution of the species of sand flies in Balochistan Province

DISCUSSION

The prevalence of active CL in boys (17-22 years) was 72.93 (1617 out of 2217 boys). However, in the school children (11-16 years) there were more cases ofactive CL (45.12 % [2643 out of 5857 children]) and in children of younger age group (5-10 years) actice CL was 44.11 % (3210 out of 7236 children). The number of children who had been exposed to the disease as evidenced by scars did not show any striking difference (52.83 % and 53.03 % respectively). Mostly children in the age group of 5-10 and 11-16

years (of both local as well as of refugee) are victimized, but non immune persons who are generally refugees and especially Government servants and laborers (including their family members) belonging to other provinces coming to the endemic areas are very much prone to contact the disease. This has happened several times that the CL has turned into an epidemic form in laborers coming from non endemic parts of the country for developmental projects like road and buildings in endemic areas. Previously, such an epidemic was reported by Lari and Khan (12) and Sanyal (21) in Uthal (Lasbella, District) where in 1977, 550 cases occurred amongst workers in a newly established Textile Mill. Control measures were instituted but 30 new cases occurred in February of 1980. The majority had multiple lesions of the wet type. A month later out of eight cases, parasites were demonstrated in two cases. *Ph. papatasi* constituted two thirds of the sand fly collection of the area. In 1980 and 1982, while conducting survey, among the textile workers of Uthal Textile Mills, Burney and Lari (16) found 29 and 60 active cases of CL all were of wet type, and in 1986, while conducting school survey in Uthal, 5 active cases all were of wet type were observed. No, dry type of lesion was recorded by them. Rab *et al.* (18) while studying prevalence of CL reported that the incidence of the disease in District Headquarter Hospital and Dispensary of Pak- Iran Textile Mills, Uthal, was approximately 1%. School survey revealed that a total of 5 out of 418 school children examined had active lesions (1.1%). In 1997 and 1999, an outbreak occurred in army personnel posted in the area of Sibi and Dhadar with 31 and eighteen cases respectively. In majority of cases observed the lesions were multiple ranging from 2 to 17 lesions, appearing mainly on face, forearms and feet.

The survey showed that in the hospitals and private clinics, the medical staff was unaware about the types of CL and they record cases of CL as “Daana “ (means a reddish papule with irritation). During the present survey, it was noticed that the local population was more concerned about other diseases common in the area, such as malaria, tuberculosis and other communicable diseases while the response to CL is very poor.

During the present investigation, eight species viz., *Ph. bergeroti, Ph. andrejevi, Sergentomyia dentata arpaklensis, S. murghabiensis, S. mervynae, S. freetownensis* var., *S. g. dryfussi turkestanica* has been found for the first time in Pakistan and *Ph. alexandri, Ph. nuri, S. babu babu, S. palestinensis, S. grekovi, S tiberiadis pakistanic, S. hodgsoni hodgsoni* has been recorded for the first time from SW Pakistan. *Ph. papatasi* (indoor=42.07%, rodent burrows=17.22%), *Ph. salehi* (indoor=3.73%, rodent burrows=2.74%), *Ph. sergenti* (indoor= 7.53%, rodent burrows= 5.29%) are known vectors of leishmaniasis in neighboring Afghanistan (11), Iran (8,20,24) and India (10). Sticky papers mostly used in the study, may be perhaps not a very useful technique for parasite isolation and live traps for catching sand flies yield better results. The best time for finding blood fed sand flies was August to October in colder areas of the province while December to February in warmer areas.

One hundred thirty nine rodents were also caught by live traps. *Rattus rattus* appeared to be the predominant species (43.88%) in the area, *Meriones* with 29.49%, *Tatera indica* with 15% and *Mus musculus* with 11.5%. None of these rodents trapped shown any clinical evidence of disease, when smears were taken from rodent ears, one *Meriones* sp. showed to harbor leishman bodies and the rest were negative. Natural infection in rodents without clinically apparent cutaneous lesions have been reported previously (3,18). However, further studies are required to incriminate reservoir host and the vector of the disease. It is also concluded that it is necessary to train and educate the health staff working in hospitals about diagnostic techniques of CL. This will help in reporting correct figures by the health staff about the numbers of CL victims in the province.

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