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Diversity of Sand Flies and Incidence of Cutaneous Leishmaniasis in Humans and Domestic Animals at Lower Dir, Pakistan

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Abstract
Cutaneous leishmaniasis (CL) in Pakistan is considered to be a serious issue, and the primary cause of this dilemma is reported through sand flies. The study focuses on the diversity of sand flies and their associated risk factors and the prevalence of CL in Lower Dir and as well as the leishmania parasites incidence in domestic pets. Three species of sand flies, *P. sergenti, P. salengensis*, and *P. major*, were captured to establish the efficacy of castor oil, mustard oil, and glycerin. A household survey of 1,388 people assessed the risk factors in relation to CL. Pets sampling determined regional prevalence rates. Better catching efficacy was reported for the mustard oil traps, which trapped the highest number of catches of sand flies. Most the identified risk factors included travel history (76.94%), a high rate of unemployment rate (46.25%), agricultural occupation (18.52%), and activities outside the household (72.33%). Most of the respondents were rural dweller and living in cement or brick houses with domesticated animals, hence exposed to the vectors. Regional analysis indicated that prevalence rates were however higher in pets compared to Timergra (65.22%) in Khal (63.08%)..

Keywords: Sand flies, castor oil, mustard oil, glycerin, cutaneous leishmaniasis, risk factors.

Introduction

Sandflies

Small blood-feeding insects belonging to the subfamily Phlebotomine are the main vectors for leishmaniasis: a complex of diseases that comprises VL (visceral leishmaniasis) and CL (cutaneous leishmaniasis).

Cutaneous Leishmaniosis in Pakistan

CL is more common in Pakistan than VL but over the whole province, especially in Baluchistan, Khyber Pakhtunkhwa, Punjab, and Sindh. This study is intended to elucidate the ecology of sand flies and epidemiology of CL in Lower Dir District, where the disease bears a critical public health burden. So far, 37 species of sand flies have been reported from Pakistan, the majority being in the genera Phlebotomus and Sergentomyia (Kakarsulemankhel, 2004). Most importantly, *P. papatasi and P. sergenti* have been identified as major vectors of the disease within this region (Rowland et al., 1999). Despite thorough reporting, no specific reports are available on the ecology of sand flies in Khyber Pakhtunkhwa, particularly in Lower Dir. Leishmaniasis is a disease which is endemic to several parts of the world causing millions of cases every year (WHO, 2010). CL, locally known as 'Kaal Dana', results in disfiguring lesions, mainly on the face, damaging the quality of life of infected people (Kassi et al., 2008). Some of the risk factors influencing CL transmission include socio-economic conditions, environmental factors, and human behavior such as traveling and outdoor activities (Akram et al., 2015).

Biology of Sand Flies

Sandflies have an average body length of about 3-5 mm, with rough coats and a characteristic posture when airborne. They have a holometabolous development lifecycle with egg, larva, pupa, and adult stages, spending most of their life under moist organic environments (Moncaz et al., 2014). Adult female members of this species feed on blood to achieve maturation of their eggs, which has led to their association as vectors for various disease-causing agents, including Leishmania species.

Medical Significance
These insects are the primary vectors of many human diseases, most notably visceral and cutaneous leishmaniasis. The population dynamics of sand flies and their ecological settings play the largest roles in transmission of leishmaniasis, possibly through environmental factors.

Cutaneous Leishmaniasis
Cutaneous leishmaniasis is one of the neglected tropical diseases that are very prevalent in Pakistan, especially in the denser populations with refugees (Afghan et al., 2011). Mainly, it is caused by Leishmania tropica in urban settings and Leishmania major in rural settings (Qamar et al., 2021). It has socio-economic impacts that could be worse based on the degree of poverty, political instability, and environmental conditions that will enhance the breeding of sand flies (Kassi et al., 2008).

Reasons for Conducted this Study
The study is on leishmaniasis and its primary vectors, which are called the sandflies in the subfamily Phlebotomine. These flies are vectors to this disease, which comprises visceral and cutaneous leishmaniasis. Just like districts of other regions in Pakistan, such as Baluchistan and Khyber Pakhtunkhwa, Lower Dir has CL as a major public health issue. Despite the high incidence of CL in the region, there exists a great deficit of detailed studies focusing on the ecology of sand flies in Lower Dir District. Vectors are essential for understanding effective control of diseases. This research study aims to fill up this research gap in the analysis of species of sand flies, specifically *Phlebotomus papatasi* and *Phlebotomus sergenti*, that act as the primary vectors of leishmaniasis in Pakistan.
This study proved to be helpful in contributing information that is valuable for formulating public health interventions by investigating these species and their role in the disease transmission pattern. The cutaneous leishmaniasis, commonly known locally as "Kaal Dana," causes disfiguring skin lesions on the face and other parts of the body, hence causing a significant deterioration of the quality of life of the residents.
Also, this disease associated with other factors such as socio-economic and environmental ones, which favor the proliferation of sand flies, and human practices, like traveling or spending a lot of their time outside, also facilitate the spread of the disease. Such a study, located in Lower Dir, would represent the worldwide scenario in that millions of infected people are seen every year. Focusing on such regions allows the understanding of both the microscopic level of the spread of disease and of any necessary outlook in line with the wider effort at the local as well as international dimensions of public health. Therefore, the study is highly important for assessing the transmission dynamics of CL in Lower Dir and strategizing about which effects can be diminished.

Materials and Methods
Case study area
Lower Dir, Pakistan is bordered by Swat to the east, Bajaur to the west, Upper Dir in the north and Malakand in the south. The tehsils that constitute the district of this region are Khall, Timergara, Balambat, Lal Qilla, Adenzai, Munda, and Samar Bagh. Lower Dir's latitude is 34.845331º N while its longitude is 71.904565º E. The entomological survey was conducted in four tehsils Timergara, Balambat, Khall, and Lal Qila. In this focused intervention, areas that were potentially at higher risk for CL transmission or had known prevalence were targeted.

Research period
The research took place between the months of August 2023 to June 2024; thus, the analysis in consideration could trace through the whole year; this way, it would possibly pick seasonal variation in CL incidence and activities of sand flies.

Confirmed cases of CL data
This involved primary data collection at treatment centers where confirmed patients are being treated for CL. The recovered data often included:

About the Patients
In general, all data: age, sex, contacts. Clinical data related to the CL infection: location and number of lesions, length of history, previous therapy. Data on travels in past months, especially to endemic regions where CL is widespread. Certain data on the position of the patient at work may give some information about possible exposure through virens or vectors.

Building Houses
Information regarding the type of abode where the patient resides, as various types of structures may be better suited to harboring sand flies, which are the main vectors for CL infection.

Risk Factors
All other risk factors associated with potential increased exposure to acquiring a CL infection, like outdoor recreation or having a shelter near natural breeding sites of sand flies.

Photography
The patients had been informed and consented before data had been collected. In this manner, the patients had been informed of the objectives behind the study, and they were willing participants. Further, photographs of CL lesions were taken for documentation and further analysis, thus bringing about pictorial evidence of the manifestations of the disease

Tools for Collection
Traps coated with sticky substance that captures sand fly as they alight to the surface. Sand fly collection was both done indoors and outdoors both during the daytime and nighttime since activity patterns of sand flies may be subject to change with environmental conditions.

Blood Sampling from pets
Blood samples were taken from domestic pets such as rats, and dogs from the study area.
The collected blood samples were serologically tested for the detection of the Leishmania parasites or antibodies, showing the presence of the parasite. Clinical indicators were assessed from the sampled animals to grasp their potential role in the transmission cycle of CL.

Ethical Issues
Permission was taken from the Ethics Committee of the University of Malakand and ethical issues have been exercised during the study.

Data Analysis
The data was input into SPSS software to analyze. Data was presented in percentages, and the prevalence rate was calculated in the studied population**.**

RESULTS
Comparison of Trapping efficacy used for the collection of sandflies Dir L. 2024.
Castor oil, glycerin and mustard oil traps proved to be maximum efficient for catching sandflies, and relative variabilities were observed in varying species of sandflies about their preference for the catching substrates (Figure 1). Castor oil caught 955 (35.87%) flies, while mustard oil caught 1855 (34.65%), and glycerin caught 1234 (35.45%) for P. sergenti. Castor oil caught 1352 (50.75%), mustard oil caught 2698 (50.42%), and glycerin caught 1756 (50.43%) flies for P. salengensis. For P. major, the numbers were 356 (13.38%) caught in castor oil traps, while mustard oil caught 797 (14.93%), and glycerin caught 492 (14.12%). In total, the majority of catches occurred in traps laced with mustard oil, and glycerin was consistent but somewhat less effective.

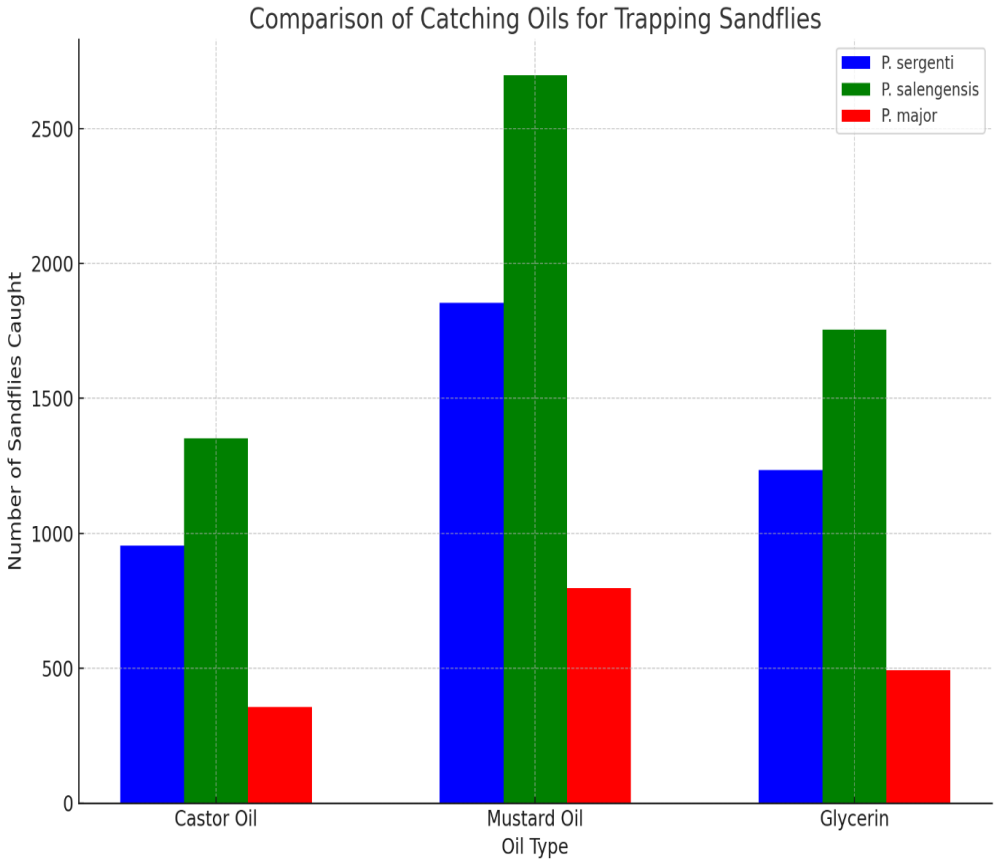

Figure 1: Comparison of Trapping efficacy used for the collection of sandflies Dir L.

Risk Factors Associated with Cutaneous Leishmaniasis in District Lower Dir.
Some of the vital risk factors associated with the transmission of Cutaneous Leishmaniasis in District Lower Dir. For instance, a questionnaire distributed to 1388 respondents showed that mobility in the previous six months was a critical antecedent whereby 76.94% of the respondents had traveled; therefore, mobility plays a critical role in disease transmission. Furthermore, 58.65% of the sample population dwelled in rural setups as CL occurs predominantly in rural settings. The prevalence of

domesticated animals in homes was highlighted by 88.76% as directly related to more cases of disease. Also, the type of materials used in housing reflected another important variable. Most homes were reported to have walls constructed from either bricks or cement (68.95%), and the ceilings were composed of concrete (66.79%), which were, therefore more contemporary. In reality, the findings were such that those who had outdoor activities had a higher chance of infection with the disease (72.33%) and those who sleep on the ground (78.96%). This shows that the risk to get CL is increased by exposure to external environments through particular dwelling conditions and personal practices.

Table 1: Risk factors associated with cutaneous leishmaniasis Dir Lower 2024.

Risk Factors	Category	Frequency	Percentage (%)
Travel History (Past 6 Months)	Yes	1,068	76.94
	No	320	23.05
Occupation	Government Employee	23	1.66
	Unemployed	642	46.25
	Agriculture	257	18.52
	Labor	108	7.78
	Others	358	25.79
Materials Used for House Walls	Bricks/Cement	431	31.05
	Stone/Mud	957	68.95
Type of Ceiling	Wood	461	33.21
	Concrete	927	66.79
Location of House	Urban	574	41.35
	Rural	814	58.65
Domesticated Animals Present in the House	Yes	1,232	88.76
	No	156	11.24
Activities Followed by Individuals	Indoor	384	27.67
	Outdoor	1,004	72.33
Sleeping Habits on the Ground	No	292	21.04
	Yes	1,096	78.96

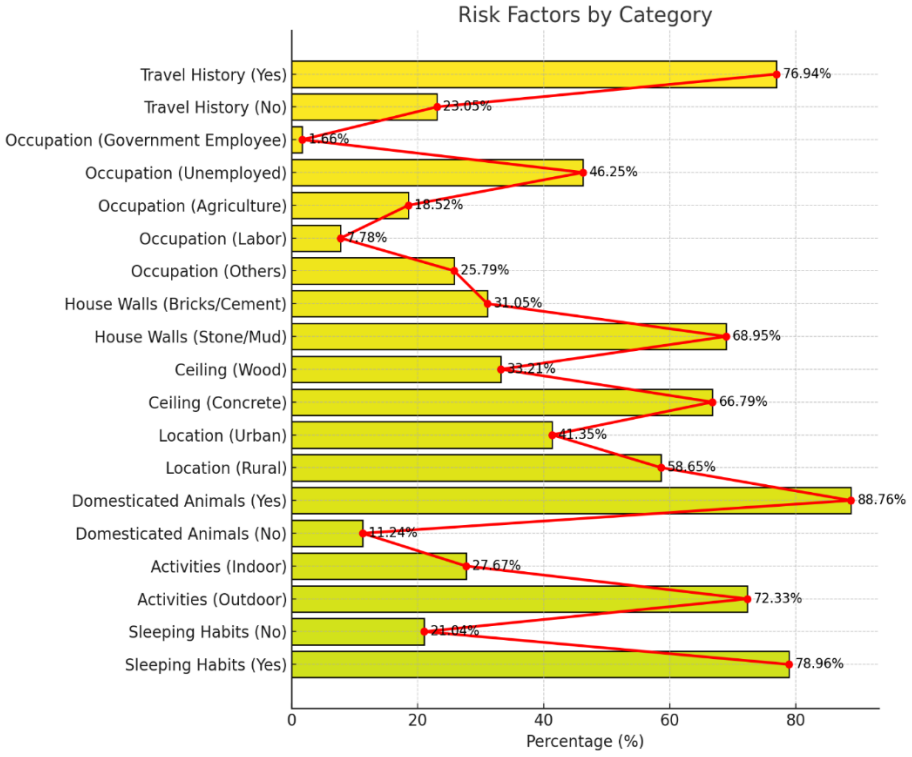

Figure 2. Risk factors associated with cutaneous leishmaniasis Dir Lower

Cutaneous Leishmaniasis Lesion Sites/Locations

This is cutaneous leishmaniasis characterized by skin wounds in the body. The major places where the wounds take place are the face, hands, and feet. The lesions vary in size and degree, from small papules to large ulcers. Figure 4 A–I give some idea of the range of these lesions, with the facial lesions in Figure 4 A and B, hand lesions in Figure 4 C and F, and foot lesions in Figure 4 D and E. Depending on where they are located, especially if the person has them on their feet, lesions can greatly

impair mobility, but lesions even on the hands can impair dexterity and function. The physical scar arising from lesions particularly on the face may also have psychological effects on the patients.

Fig A **Fig B** **Fig C**

Fig D **Fig E** **Fig F**

Fig G **Fig H** **Fig I**

Figure 4. (A, B, C, D, E, F, G, H and I) Showing different type of lesions on face, hand and feet, in the patients of CL in Dir lower, Pakistan.

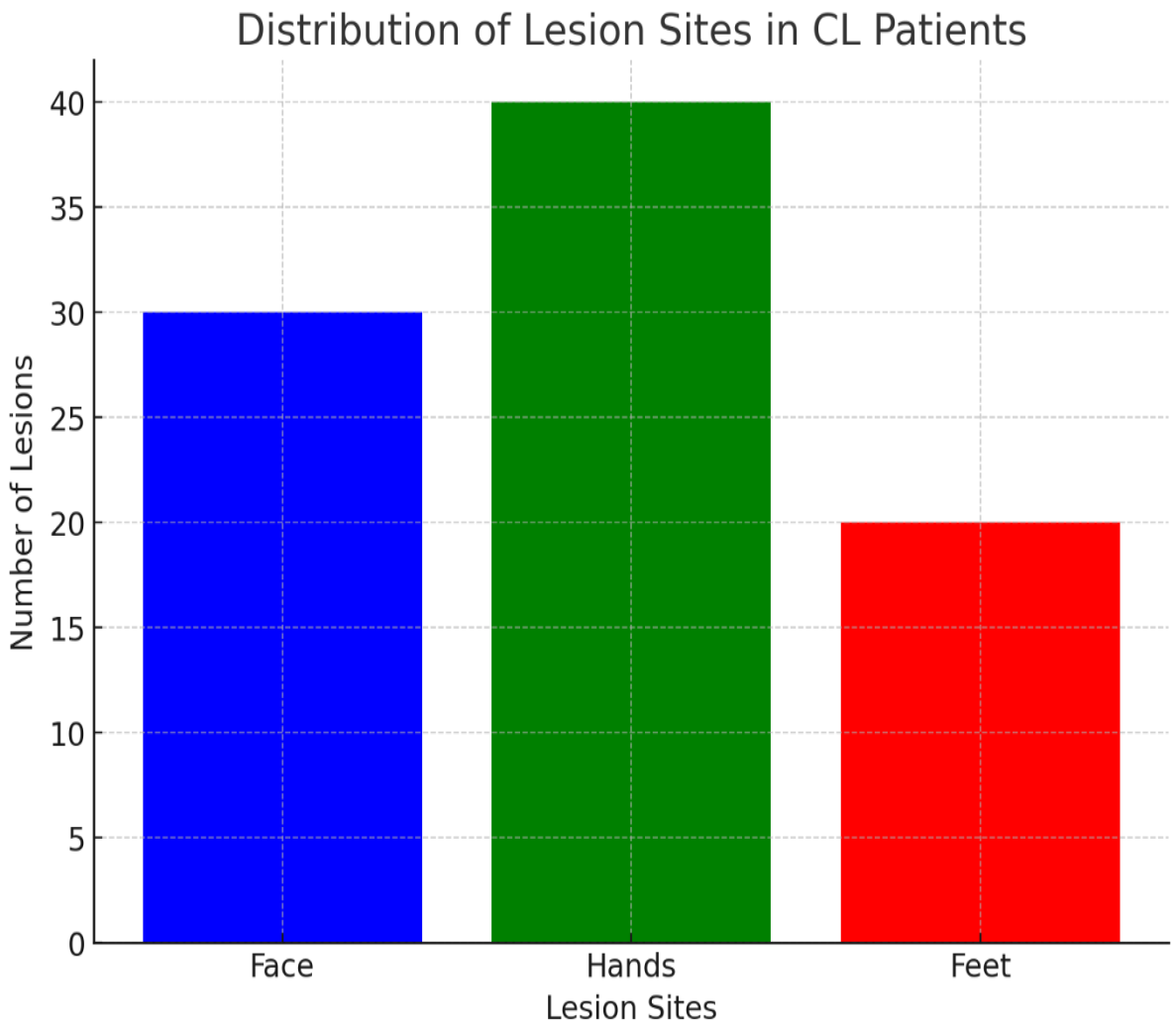

Figure 3: CL lesions can appear anywhere on the body

Prevalence of Leishmania Parasite in Domestic Pets in District Lower Dir 2024.

This study also involved assessing the prevalence of the Leishmania parasite among the pets at home, including rats and dogs. This was in the different tehsils of District Lower Dir. The prevalence rates were highly significantly different by region. The highest prevalence existed in Timergra with 65.22%, and Khal, with 63.08%, which means these places are important reservoirs for the disease. Lower prevalence rates were documented in Maidan (37.93%), Adenzai (28.13%), Samrbagh (29.41%), and Balambat (18.18%).

Table 2: Prevalence and positive cases of Leishmania in the blood of Domestics Pets Dir L.2024

Tehsil	Host Reservoirs	Positive samples	Prevalence (%)
	Rats and Dogs		
Khal	65	41	63.08%
Timergra	23	15	65.22%
Maidan	29	11	37.93%
Adenzai	32	9	28.13%
Samrbagh	17	5	29.41%
Balambat	11	2	18.18%

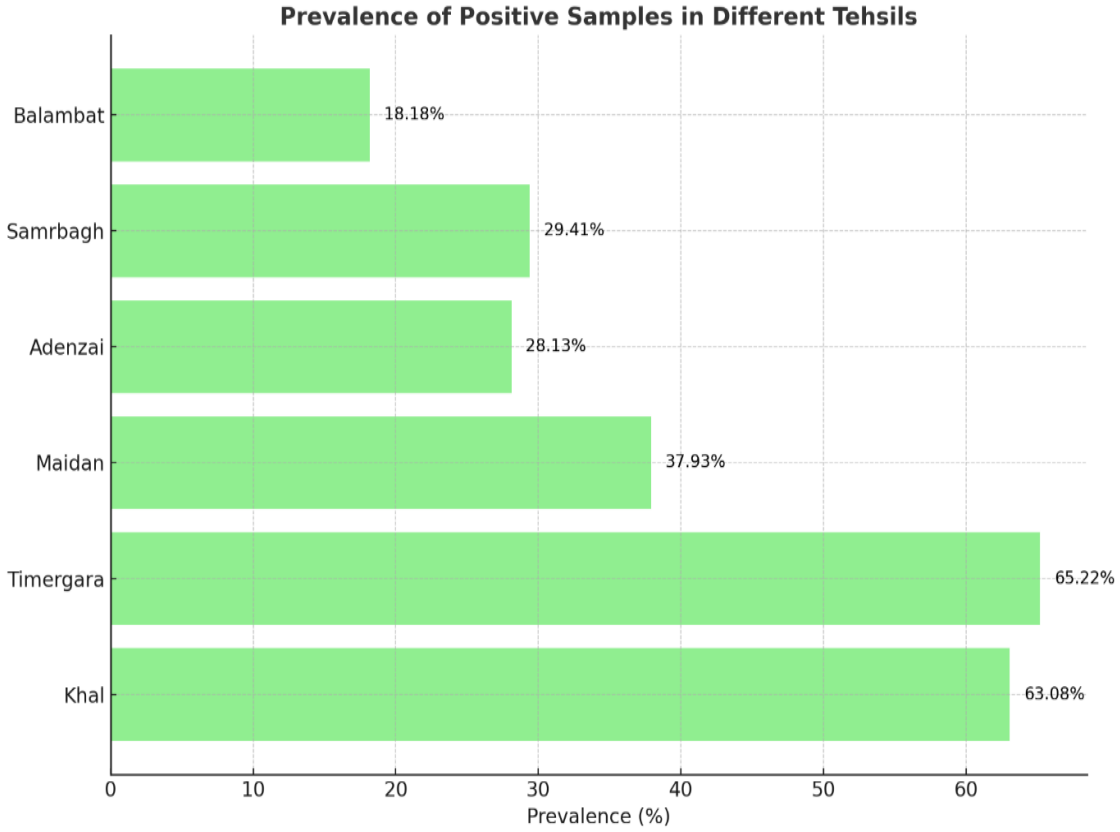

Figure 5. leishmania parasite detected in the blood of domestic pes Dir Lower 2024.

DISCUSSION

From the study, Phlebotomus palingenesis dominated at the entraps with 50.43%, followed by P. sergenti that recorded 35.45%, and P. major was seen with 14.12%. The Mustard Oil Trap had the largest capture rate, then Glycerin, and finally Castor Oil Traps. In light of data obtained, it would then be said that even if P. salengensis is predominantly high for most periods, the differences in capture rates among these different traps must mean difference in trap efficiency or species preference. Ali et al. (2016) also reports similar findings with *P. salengensis* (42.2%) and *P. sergenti* (36.6%) dominating the trapped sandflies from Dir districts, but variations in species distribution would best be attributed to localized ecological differences, trap types or seasonal events. It also emphasizes the need for continued checks on trap efficiency and species-specific preferences in order to enhance surveillance and control measures.

The questionnaire gave significant epidemiological insights. The greatest percentage of 76.94% of patients had travelled recently, which may expose them to various endemic regions and, consequently, be exposed to the causative agents of the disease. The majority of the individuals in this study were unemployed (46.25%), which was accompanied by various occupations like agriculture and laborers. This condition may also imply socioeconomic involvement in the exposures associated with the disease.

The most used housing materials in the area of study were bricks/cement walls (68.95%) and concrete ceilings (66.79%), contrasting findings in other regions, for example, Bolivia, where it was so common to have walls made of wood or plastered mud. There is a high proportion of patients from rural areas (58.65%) compared with patients from urban areas, which accords with Kassiri et al. 2014, that found results from Iranian research concluding that there is a higher risk of sand-fly exposure in rural settings.

This high percentage of patients who have domesticated animals (88.76%) indicates that animals may be an essential facilitator in the transmission mode of sand flies. The higher frequency of outdoor activities by patients accounted for 72.33%, and the low sleeping outdoors rate of 21.04% further point towards exposure pathways and practices that may enhance increased risks of diseases.

The authors of the study on the report, however disagree with Iqbal et al. (2022), who reported a more significant percentage of patients without any travel history. This inconsistency may be due to variation in travelling, disease dynamics, or both. There is a deviation of occupational prevalence of CL in our work and Rehman & Hidayat (2017) reported higher prevalence of CL among shepherds. The heterogeneity may refer to the differential risks at local level or occupational exposure. The housing construction materials and types of ceilings used in our study were different from those reported by Eid et al. (2018) in Bolivia, suggesting that local building practices may influence the habitat and exposure risks for sandflies. The higher proportion of patients living in urban areas in our study is contrary to the finding by Kassiri et al. (2014), suggesting changes in dynamics of urban-rural distributions of sandflies and CL.

Pet samples were examined and found to have regional variations in terms of prevalence rates across tehsils. The highest rates can be seen in Khal and Timergra with the implication that it is more widespread in these areas. On the other hand, from one tehsil to another, with increasingly lower prevalence rates, one finds Adenzai, Samrbagh, and Balambat. This calls for

segmented interventions and disparate surveillance approaches. These areas need intensified control measures as well as community education. Proactive surveillance in these lower-prevalence areas like Adenzai, Samrbagh, and Balambat would help monitor these trends and institute early interventions to prevent their increase.

Conclusion

The present study conducts an extensive investigation in sandfly diversity as well as risk factors related to CL and prevalence at Dir Lower, Pakistan. It focuses on Phlebotomus salengesis as the primary species, asking for appropriate trapping techniques to study their activities. The study has highlighted that recent travel, outdoor activities, and contact with pets are some of the major transmission routes of CL. Housing materials and housing conditions also determine habitats for sand-flies. Areas with high prevalence call for intensified control activities along with education among the community. In other words, effective CL management would thus require the simultaneous intervention of three significant dimensions: environmental, occupational, and behavioral. Further research with a multidisciplinary tailored approach to public health interventions is important for the diminution of CL burden and attainment of healthy communities.

Author Contributions:

Conceptualization, M.A., A.U., K.K., A., and F.K.; Methodology, M.A., A.U., K.K., A., and F.K.; Software, I.I.; Validation, F.K. and I.I.; Formal Analysis, M.A., S.Y., K.K., A., and F.K.; Investigation, F.K. and I.I.; Writing—Original Draft Preparation, M.A., A.U., K.K., A., and F.K.; Writing—Review and Editing, F.K., I.I., S.Y., and S.S.; Supervision, M.A., F.K., S.Y., and S.S.; Project Administration, M.A. and F.K.

All authors contributed equally to the development and writing of this manuscript and have read and agreed to the published version

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Field Questionnaire for Sand Flies

Questions	**Options/Answers**
Materials used for house walls	0: Stone/mud 2: Bricks/Cement 3: Others (Please specify): _____
Type of ceiling	0: Concrete 1: Wood (beam) 2: Wood (thatched) 3: Others (Please specify): _____
Location of the house	0: Village/Rural 1: Urban
Vegetation in or near the house	0: No 1: Yes
Domesticated animals present in home	If yes, list types and number: _____
Activities followed by individuals	0: Indoor 1: Outdoor
Sleeping habits on ground	0: Yes 1: No
Where do you sleep in your house	0: Indoor 1: Outdoor
Is there proper sewage system in your locality?	0: No 1: Yes
Did other cases occur in your home after the first one?	0: No 1: Yes
What do you think is the causing agent of the disease?	Your answer: _____
Do you know about sandfly?	Yes / No
What medicine or treatments did you use for a cure?	Your answer: _____
What preventive measures do you suggest for protection from Cutaneous Leishmaniasis?	Your answer: _____

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