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Prevalence of Cutaneous Leishmaniasis and Identification of Vector Phlebotomus Species Leishmania in Tehsil Adenzai Dir L. Pakistan

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Abstract

This study investigates the prevalence and distribution of Cutaneous Leishmaniasis (CL) in Tehsil Adenzai, District Dir Lower, Khyber Pakhtunkhwa, from August 2023 to February 2024, reporting a total of 1388 cases. The findings revealed a notable gender disparity, with males (82.95%) being predominantly affected, compared to females (18.05%), likely due to higher outdoor exposure and occupational risks. Age-wise, the highest prevalence was observed among individuals aged 0-15 years (36.17%). Geographically, the hotspots were identified as Badwan and Chakdra, with 432 and 341 cases, respectively. Lesion analysis indicated that most patients presented with dry lesions (76.15%), and the face was the most commonly affected site (37.78%). Risk factors such as recent travel history (76.94%), rural residence (58.65%), and proximity to domesticated animals (88.76%) were significantly associated with CL transmission. Moreover, species distribution patterns showed *Leishmania tropica* as the most prevalent, followed by *Leishmania major* and *Leishmania infantum*, each exhibiting distinct geographical and occupational predilections. The results highlight the urgent need for targeted interventions, especially for high-risk groups, through enhanced vector control, community education, and improved housing conditions to mitigate CL transmission.

Key Words: Cutaneous Leishmaniasis, Risk factors, Leishmania parasites, Tehsil Adenzai,

Introduction

Cutaneous Leishmaniasis (CL) is a significant public health issue in various regions of the world, especially in countries like Pakistan (World Health Organization, 2023). The disease occurs in two main forms, namely anthroponotic and zoonotic (Reithinger et al., 2007). Most transmission of anthroponotic CL (ACL) is between humans, whereas zoonotic CL (ZCL) typically involves an animal reservoir, such as rodents, and is primarily caused by *Leishmania major* (Alvar et al., 2012). Along with visceral leishmaniasis (VL) and mucocutaneous leishmaniasis (MCL), CL is one of the main forms of leishmaniasis, affecting millions worldwide, with more than 20 identified species of *Leishmania* known to infect humans (World Health Organization, 2010).

In Pakistan, CL is reported in all four provinces: Khyber Pakhtunkhwa (KP), Baluchistan, Punjab, and Sindh (Rowland et al., 1999). The disease burden is notably higher in rural and economically deprived settings, which is linked to poor living conditions and neglected healthcare infrastructures (Kolaczinski et al., 2004). Afghan refugee camps in KP and Baluchistan have become one of the main foci of the disease due to high population density and lack of resources (Rehman et al., 2018). Recently, the southern parts of KP alone reported about 28,000 cases (Khan et al., 2022). The predominant species in this setting is *Leishmania tropica*, while *Leishmania major* is mainly found in rural areas (Mansoor et al., 2016). Zoonotic cutaneous leishmaniasis is rampant in the merged tribal areas of KP, known previously as FATA, where an animal

reservoir for the parasite exists (Nasir et al., 2016).

Leishmaniasis is endemic in more than 98 countries worldwide and poses a threat to around 350 million people, with 1.5 to 2 million new cases reported annually (Alvar et al., 2012). The annual cases of CL alone span 600,000 to 1 million cases, while VL, the more virulent condition, affects 50,000 to 90,000 people per year (Desjeux, 2004). In Pakistan, reported yearly cases vary from 21,000 to 35,000, depending on the region (World Health Organization, 2021). Higher incidence rates correspond to areas with climatic and environmental conditions that favor the breeding of sandflies, such as arid and semi-arid zones (Rehman et al., 2018). Human migrations, deforestation, urbanization, and economic instability also play significant roles in the incidence of CL in Pakistan (Jadoon et al., 2015).

Geographical and socio-economic factors influence the prevalence of CL in Pakistan. The most important contributors to its spread include poverty, rural residence, lack of health infrastructure, and a large influx of Afghan refugees (Bashir et al., 2020). Human behavior, such as outdoor work and sleeping without protective measures, increases exposure (Memon et al., 2021). Environmental conditions such as temperature, humidity, and rainfall affect the breeding and activity of sandflies, thereby impacting the spread of the disease (World Health Organization, 2010). Additional factors such as migration, urbanization, and deforestation interfere with the natural ecology of the sandfly, exposing the human population to increased vector-human contact (Rowland et al., 1999).

Leishmaniasis exists in three major forms: Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (MCL), and Visceral Leishmaniasis (VL) (Alvar et al., 2012). CL, locally referred to as "Kaal Dana" in Pakistan, causes extensive scarring of the skin and is mainly found on the face and other exposed surfaces (Reithinger et al., 2007). It is the most common form in the country, showing a biased prevalence among women and children in Pakistan and Afghanistan (Kolaczinski et al., 2004). It contrasts with the major urban form, *Leishmania tropica*, which is more frequent compared to the minor, rural form, *Leishmania major* (Mansoor et al., 2016).

Visceral Leishmaniasis (VL) is much more serious and even lethal when untreated. It is mainly restricted to the northeastern part of Pakistan, especially in Azad Jammu and Kashmir, as well as Gilgit-Baltistan (Nasir et al., 2016). Dogs are considered the major reservoir of *Leishmania infantum*, which is believed to be the causative species of VL in this region (World Health Organization, 2023). Though certain species of sandflies like *Phlebotomus argentipes* are known to be primary vectors in other parts of the world, their role in VL transmission in Pakistan is not well elucidated yet (Alvar et al., 2012).

Sandflies also transmit other diseases, such as bartonellosis and sandfly fever. Bartonellosis is caused by the bacterium *Bartonella bacilliformis* and is mainly found in areas such as Peru, Ecuador, and Colombia, where *Lutzomyia* sandflies dominate (Kaufman & Rutz, 2012). Bartonellosis has two forms: acute Carrion's disease, or Oroya fever, and chronic Verruga peruana (Jacobson et al., 2003). Sandfly fever, a phlebovirus-caused disease, is mainly reported among military and travel

populations in endemic countries in Asia and the Mediterranean (Desjeux, 2004).

There is a significant lack of updated data related to the burden of leishmaniasis in this region (World Health Organization, 2021). This study aims to conduct a comprehensive surveillance of CL in Tehsil Adenzai, Lower Dir, with an emphasis on identifying commonly associated risk factors. The research will also map the geographical spread of the disease and ascertain which species of *Leishmania* prevail in the region (Jadoon et al., 2015). This study aims to fill these gaps and provide valuable insights into the epidemiological patterns of CL in this region, thereby aiding in the development of targeted strategies for disease management (Bashir et al., 2020). An improved understanding of species-specific transmission dynamics will also contribute to the formulation of effective treatment protocols and prevention methods.

Research Methodology

Tehsil Adenzai

Tehsil Adenzai is centrally located in the District Malakand. It lies in the north of District Swat which falls on the right bank of the Swat River. Badwan falls in its north, Pengal to its west, Talash to its east, and Batkhila to its south. Major Business Center Chakdra is the major business center of this tehsil. It has concrete houses which are available in the region with a dense population. Houses of mud or iron sheets can be seen everywhere in rural areas. Flora and fauna abound in this area, but the species diversity cannot be accessed since there is no organized data available.

Study Duration

This study was done for six months, from August 2023 to February 2024.

Data Collection

326 confirmed cases of CL were recorded during the study, which was reported from health facilities spread all over Tehsil Adenzai, including THQ Chakdra, CD Badwan, RHC Gulabad, RHC Ouch, CD Khairabad, and BHU Pengal.

Data Collection Procedure;

Data collection was done with the use of a structured questionnaire that includes variables like patient demographics, lesion characteristics, environmental factors, and preventive measures. Informed consent was taken, and pictures of the lesion were taken with the help of an Android phone.

Study Design

Patients clinically suspected of having CL were selected; spots from a patient's lesion on the filter paper exudate were collected.

Blood Sample Collection

The samples were preserved in polyethylene bags with silica gel beads that prevent moisture contamination and were kept at 4°C in a refrigerator. This type of method prevents loss of sample integrity for further lab work and species identification.

Data Analysis

Data were entered and analyzed on Microsoft Excel considering variables like gender, month, age, area, and lesion characteristics, and using Chi-square tests for the identification of significant risk factors associated with the prevalence of CL in Tehsil Adenzai.

Results

The study reveals a detailed analysis of Cutaneous Leishmaniasis (CL) prevalence based on gender, age, geographical area, and lesion characteristics. Gender-wise, males were disproportionately affected, with 82.95% of the total cases, likely due to higher occupational and outdoor exposure, while females accounted for

18.05%. Monthly distribution data indicates that the highest number of cases occurred in September 2023, accounting for 20.50% of the total, with a gradual decline observed until February 2024. Age-wise, the highest prevalence was found in children aged 0-15 years (36.17%), indicating increased vulnerability in younger populations. Geographically, hotspots like Badwan and Chakdara showed the highest concentration of cases, suggesting localized clusters of disease transmission. The face was the most common site of lesions, accounting for 37.78%, possibly due to increased exposure.

Species distribution further highlights *Leishmania tropica* as the predominant species, with 73 samples, primarily affecting males and various occupational groups such as government employees and laborers. *Leishmania major* was relatively less common, found mainly in unemployed individuals and in areas such as Tawda Chena and Chakdara. *Leishmania infantum* was even rarer, with 12 cases spread among laborers and residents of Badwan and Gul Abad. These findings emphasize that each species shows distinct demographic and geographical patterns, underlining the need for targeted interventions. The data suggests that public health efforts should focus on high-risk areas and vulnerable groups, using integrated strategies like vector control, community education, and enhanced surveillance.

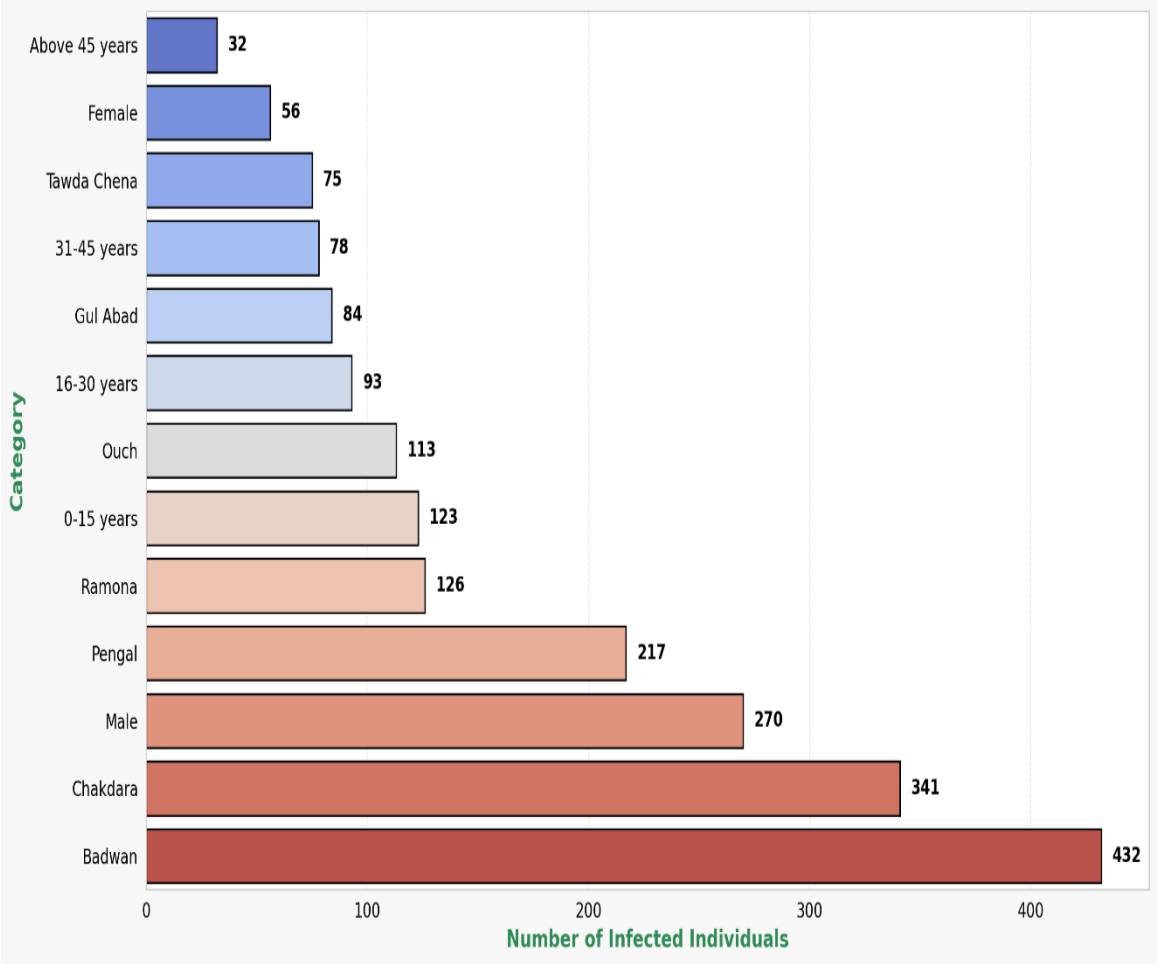

Fig 1 CL Prevalence Data in Tehsil Adenzai District Dir Lower, Kp

Table 1 Cutaneous leishmaniosis Prevalence in Tehsil Adenzai District Dir Lower, 2023-2024

Category	Sub-category	Infected Individuals	Prevalence (%)
Gender-wise Distribution	Male	270	82.95%
	Female	56	18.05%
	Total	**326**	**100.0%**
Month-wise Prevalence	August 2023	59	18.49%
	September 2023	67	20.50%
	October 2023	55	16.80%
	November 2023	45	12.80%
	December 2023	34	10.72%
	January 2024	46	14.31%
	February 2024	20	6.63%
	Total	**326**	**100.0%**
Age-wise Prevalence	0-15 years	123	36.17%
	16-30 years	93	20.24%
	31-45 years	78	25.58%
	Above 45 years	32	18.01%
	Total	**326**	**100.0%**
Area-wise Prevalence	Badwan	432	31.12%
	Chakdara	341	24.57%
	Pengal	217	15.63%
	Ramona	126	9.08%
	Ouch	113	8.14%
	Gul Abad	84	6.05%
	Tawda Chena	75	5.41%
	Total	**1388**	**100.0%**
Type of Lesion	Dry	250	76.15%
	Wet	85	34.85%
	Total	**326**	**100.0%**
Number of Lesions	Single	140	43.45%
	Double	107	32.70%
	Multiple	79	24.85%
	Total	**326**	**100.0%**
Location of Lesions	Face	123	37.78%
	Upper Limbs	96	29.91%
	Lower Limbs	75	22.72%
	Others	32	9.90%
	Total	**326**	**100.0%**

Cutaneous Leishmaniasis (CL) cases reveal significant gender-based disparity, with males accounting for 82.95% (270 cases) and females 18.05% (56 cases) of the total 326 reported cases. Monthly prevalence indicates the highest number of cases in September 2023 (67 cases, 20.50%) and the lowest in February 2024 (20 cases, 6.63%). Age-wise, children aged 0-15 years are the most affected (123 cases, 36.17%), followed by adults aged 31-45 years (78 cases, 25.58%), and individuals above 45 years show the lowest prevalence (32 cases, 18.01%).

Geographically, hotspots such as Badwan (432 cases, 31.12%) and Chakdra (341 cases, 24.57%) exhibit the highest prevalence, indicating clustering in these areas. Lesion-type analysis shows that dry lesions are more common (76.15%), and most patients present with single lesions (43.45%).

Anatomically, the face is the most frequently affected site (37.78%), followed by upper limbs (29.91%) and lower limbs (22.72%). These patterns suggest that exposure-related factors, including geography and outdoor activities, play a critical role in disease distribution.

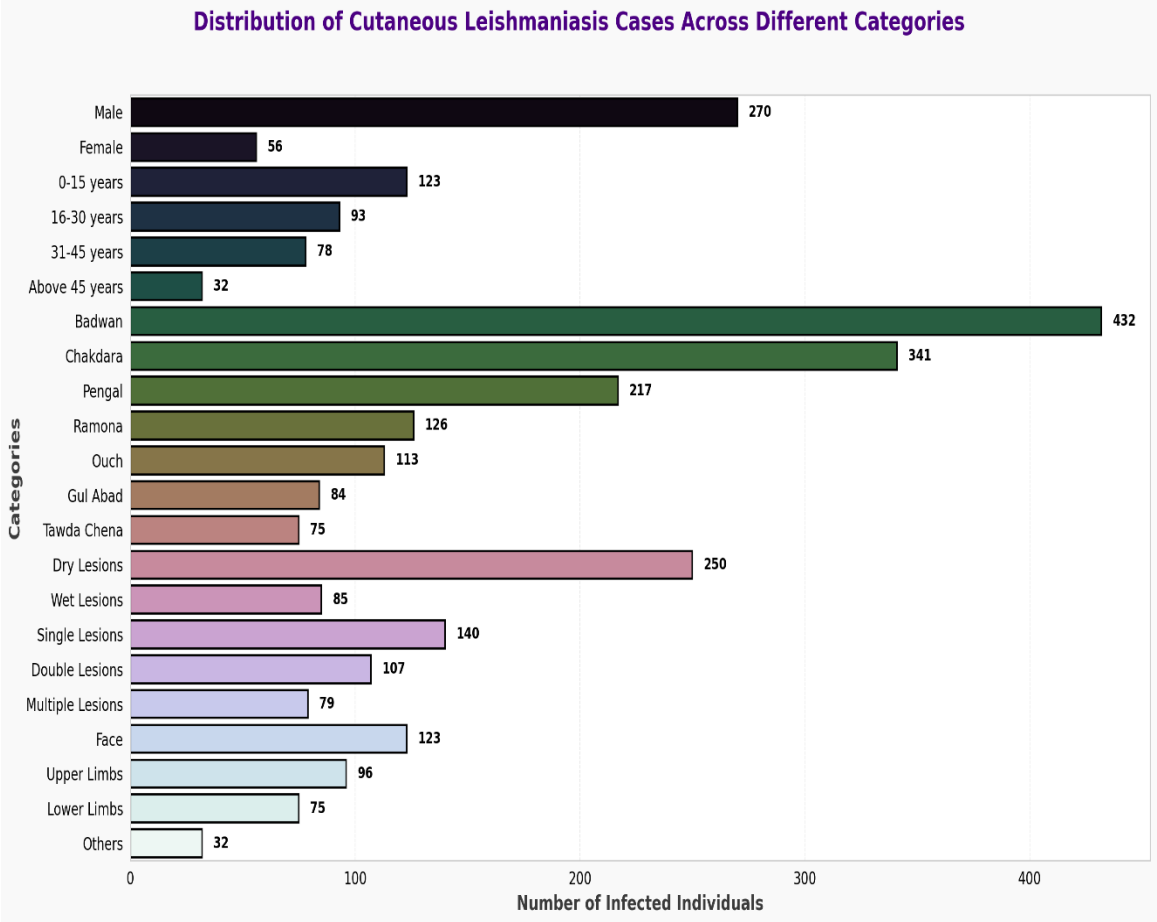

Figure2 Risk Factor Analysis of Cutaneous Leishmaniasis

Figure 3: Blood sampling for detection of Leishmania in Cutaneous Leishmaniosis Patients in Tehsil Adenzai Dir lower

Table 2 The distribution of species of Leishmania in District Dir lower 2024.

Species	Category	Type	Total Samples
Leishmania tropica	Gender	Male	50
		Female	23
	Age Group	Above 45	23
		Under 15	21
		15-30	15
		31-45	14
	Area	Tawda Chena	25
		Gul Abad	21
		Badwan	15
		Chakdara	12
	Occupation	Government Employee	16
		Laborer	16
		Unemployed	16
		Agriculture	13
		Business/Private	12
Leishmania major	Gender	Male	10
	Gender	Female	5
	Age Group	Under 15	6
		31-45	4
		15-30	4
		Above 45	1
	Area	Tawda Chena	7
		Chakdara	4
		Gul Abad	2
		Badwan	2
	Occupation	Unemployed	5
		Business/Private	5
		Government Employee	2
		Agriculture	2
		Laborer	1
Leishmania infantum	Gender	Male	8
		Female	4
	Age Group	31-45	5
		Above 45	3
		15-30	3
		Under 15	1
	Area	Badwan	4
		Gul Abad	4
		Chakdara	2
		Tawda Chena	2
	Occupation	Laborer	4
		Agriculture	3
		Unemployed	3
		Government Employee	2

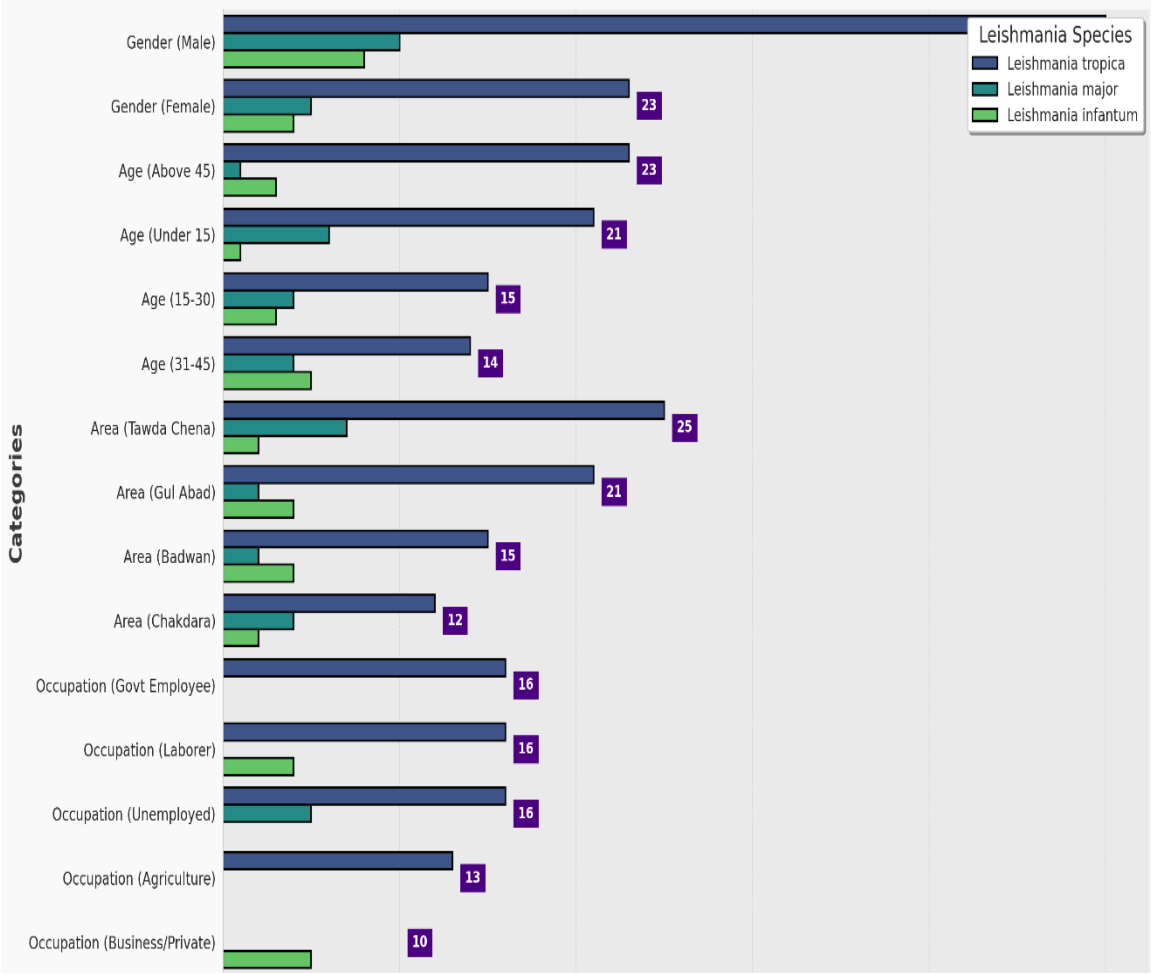

Fig 4 Distribution of Leishmania Species

Leishmania tropica is far more common in men, at 50 cases, compared to women, at 23. In Leishmania major, again males have twice as many cases as females, 10 cases in men, and 5 in females, a consistent trend. Leishmania infantum does the same at 8 cases among males and 4 among females. This trend may be attributed to higher outdoor and occupational exposure that is often experienced among the males as they carry out activities that improve their exposure to vector-exposed environments.

Relatively evenly distributed, except for the highest frequencies in the "Above 45" category which reaches 23, and a very significant number of cases for the "Under 15" group with 21 cases. In Leishmania major, the higher number of cases also seems to be predominantly within the younger population, particularly under 15 years old with 6 cases, which gradually diminishes with age, indicating that there does exist vulnerability in the younger populations. In comparison, L. infantum was dispersed more uniformly with an extreme value in the "31-45" age group of "5 cases" and a minimum value of "1 case" within the "Under 15" age group. The distribution exhibits that increased exposure over time makes older adults vulnerable to the infection by L. tropica whereas L. major has more potential among the young people due to their behavioral practices as well as an

underdeveloped immune system.

Geographical spreading, Leishmania tropica is concentrated in the region of Tawda Chena with 25 cases and Gul Abad with 21 cases, followed by Badwan with 15 cases and Chakdara with 12 cases. This means that the regions of Tawda Chena and Gul Abad are potential hotspots for L. tropica transmission. Cases of Leishmania major are reported mainly from the Tawda Chena with 7 cases, lesser numbers in Chakdara with 4 cases, and only 2 cases reported from Gul Abad and Badwan that outbreaks are localized. Cases of Leishmania infantum, too also reveal higher frequency in Badwan and Gul Abad with 4 cases each, and a lesser number of cases in Chakdara and Tawda Chena with 2 cases each. These regional patterns suggest that each species occupies a different ecological niche and has differential patterns of transmission that are likely explained by local variations in environmental conditions and vector availability.

Leishmania tropica was found with equal distribution over several occupational categories, that is, Government Employees, Laborers, and the Unemployed each corresponding to 16 cases, followed by Agriculture (13 cases) and Business/Private (12 cases). This would imply an exposure that is broad occupational rather than occupation-specific activity and may be more associated with environmental factors. Leishmania major is most common in the Unemployed and Business/Private occupations, at 5 cases each, and relatively fewer in Government Employees, Agriculture, and Laborers, indicating much less occupational dispersion. For Leishmania infantum, the most affected are the Laborers with 4 cases, followed by Agriculture with 3 cases, while the Unemployed and Government Employees have fewer cases, at 3 and 2, respectively. The diverse occupational distribution may suggest that L. tropica has a more extended occupational range, and L. major and L. infantum are mainly dominated by specific environment or behavior factors.

Figure 5. Showing Sampling from spots of the lesions and microscopic view of the parasites CL in Adenzai, Dir Lower, KP, Pakistan

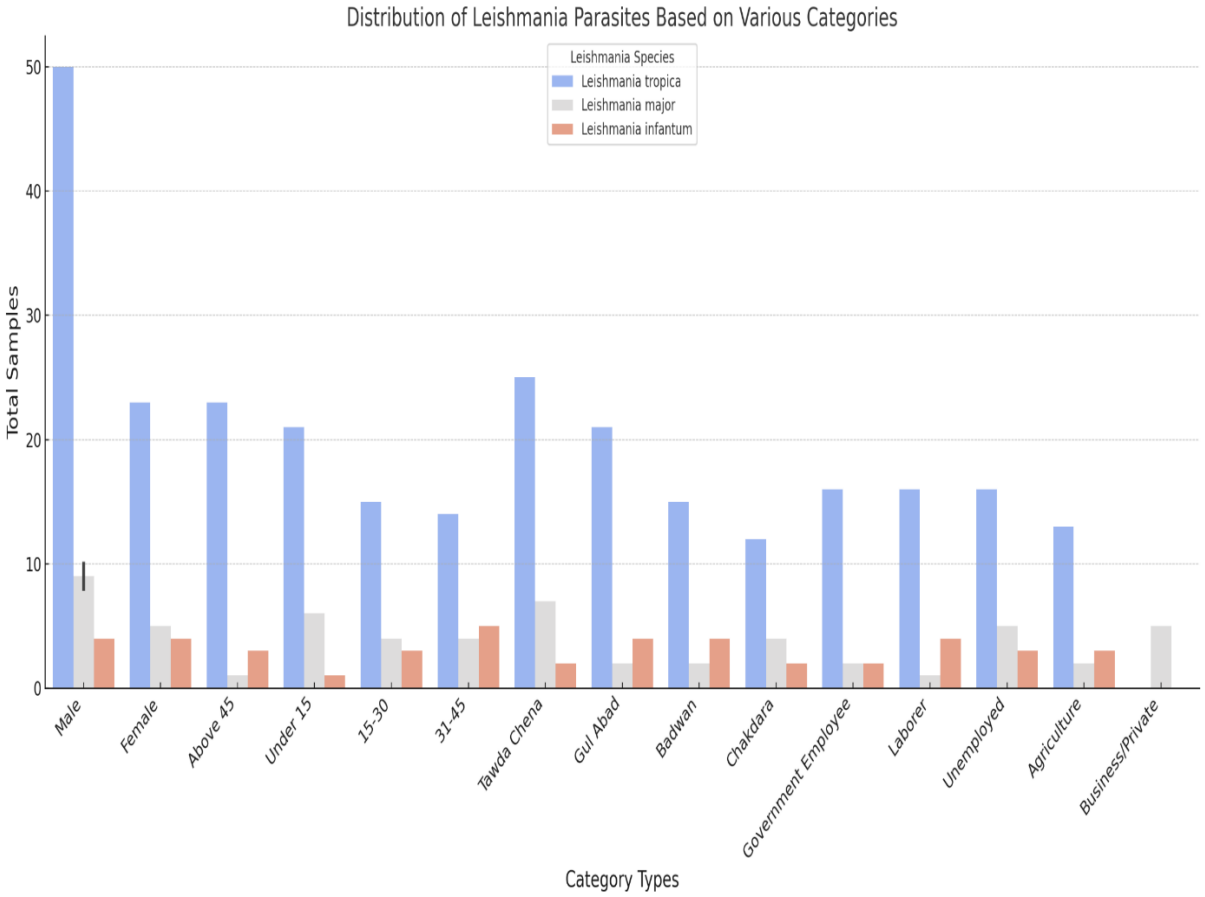

Figure 6 Distribution of Leishmania Species by Gender, Age Group, Geographical Area, and Occupation 2024 Dir L tehsil Adenzai

Discussion

Cutaneous Leishmaniasis is one of the vector-borne parasitic diseases considered to be wide across the world and based on very evident clinical and epidemiological diversities. It increases everywhere in the world and mostly poses a serious public health problem in a certain area. CL spreads throughout disparate regions of Baluchistan and Khyber Pakhtunkhwa, with reported outbreaks in Sindh and Punjab, writes Rahman et al., 2003; Khan et al., 2005.1388 patients suffering from active leishmanial skin lesions were identified in the present study. This indicates males had a higher prevalence of CL. Compared to females, which does support findings studied in district Malakand, Muzaffargarh, and abroad in Iran. This can be attributed to the fact that males spend more time outside, and subsequently have increased contact with sand-fly bites and have lesser coverage due to clothing. Travel patterns, with the majority of males often outside working, contribute to this gender-based difference.

Maximum prevalence was in September 20.5%, August 18.49%, and October 16.8%, unlike studies from Northern Pakistan, Iran, and India where high prevalence was reported in the summer months of the year. This deviation can be accredited to the fact that sandflies are relatively highly active in the warm months. Climatic factors, especially temperature and humidity, affect the reproduction of sandflies. A remarkable increase in prevalence was noticed within the 0-15 years age group. While a remarkable decline was observed within individuals above 45 years, in the present study. Similar trends have been recorded from Dir, Kohat, and elsewhere, which can be attributed to increased outdoor activities and underdeveloped immune systems in children. Our study found the differences in prevalence to range as high as 31.12% in Badwan and as low as 5.41% in Tawda Chena. As with the previous studies, this conclusion verifies CL as endemic within Dir Lower and indicates possible causes for these regional differences. Dry-type lesions outnumbered the wet-type lesions; such observation accorded well with investigations conducted in the Dargai Kurram agency and Iran. The higher prevalence of dry-type lesions might be associated with Leishmania tropica causing anthroponotic cutaneous leishmaniasis, ACL, as reported by Marco et al. 2006.

The prevalent lesion was in the form of solitary lesions. Consistent with findings in Dir and Karak, lesions are caused by the physiological behavior of the sandflies. The lesions are most predominant on the face, upper limbs, and lower limbs as most body parts are exposed while resting and sleeping. (Sharif et al., 2017; Nawaz et al., 2010; Aara et al., 2013). Our study had more patients who had a history of travel compared to those who had never traveled. This could be an indication that traveling has some association with the health condition under study.

Iqbal et al. (2022) reported that in district Mardan, a substantial number, 60.7% of the patients, had no travel history. Geographical and socio-economic differences might have caused a skewed distribution of travel history

across the study areas. In our study, the majority of the patients were unemployed, followed by agricultural labor. The occupational pattern would be one of the factors to influence transmissivity or prevalence of health conditions.

According to Rahman and Rehman, while investigating at Dir, a completely different pattern of occupations emerged where shepherds were at the highest prevalence of the condition, thus suggesting that the risk factors for health conditions may vary according to different occupations and regions. I have found that brick/cement dominates the wall material, unlike in the case of Eid et al. (2018) study in Bolivia, where dominance by wood or plastered mud is evident. Such differences can be attributed to regional building practices, climate, and resources available among others.The type of ceiling too, varied, with concrete dominating my study compared to Eid et al. (2018) where wood was more common. These could be attributed to regional construction patterns and material supply. This study reported a higher percentage of patients in urban compared with rural areas. Contrarily, Kassiri et al. (2014) study in Iran reported a higher percentage of patients from rural areas. Such a difference might result from factors such as population density, access to health facilities, and environmental conditions. We had noticed, in the course of our investigation, that the domesticated animals were always found within their dwellings. This is in agreement with the observation of Jamal et al. (2013) at district Dargai, wherein a significant percentage of the patients kept the animals. This gave an indication that the health condition being investigated might have some association with the presence of the animals. The study showed that most of the subjects were engaged in outdoor activities, in agreement with the survey reported by Numan et al., 2022. This may probably hint at common ways of living or culture practiced in the population under study.

Author Contributions

Ihsan Ullah led the initial data collection and wrote the first draft of the manuscript. **Fawad Khan** (corresponding author) managed the research design and overall execution of the study. **Dr. Mahnoor Pervez** reviewed the research methods and helped improve the study's accuracy. **Samina Yasmin** conducted a detailed literature review and helped refine the manuscript. **Bahar Uddin** validated the data and coordinated field activities. **Shahab Saqib** handled statistical analysis and species identification. **Sana Shahid** managed lab work and sample processing to ensure data quality. **Faseeha Ilyas** oversaw project administration and created visuals to clearly present the findings. **Muhammad Usman** supported field surveys and data organization. **Inam Ullah and Abdul Latif** developed the project idea and reviewed the final manuscript, providing valuable insights that strengthened the study.

Conflict of Interest

All authors declare no competing financial, professional, or personal interests.

How to Cite

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