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EPIDEMIOLOGY OF *LEISHMANIA TROPICA* AND *LEISHMANIA INFANTUM* INFECTIONS IN DISTRICT CHITRAL FOR THE YEAR 2021-2022



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Abstract

This study presents a detailed epidemiological analysis of Leishmania Tropica and Leishmania Infentum infections in the Chitral District for the years 2021 and 2022, focusing on gender disparities, seasonal peaks, and regional hotspots.

Objectives: *The primary objectives were to determine the infection patterns of L-Tropica and L-Infentum across different demographic groups, seasons, and regions, and to identify the potential environmental and socio-cultural factors contributing to these trends.*

Methodology: *A retrospective analysis was conducted using case data from health centers in the Chitral District. The study examined infection rates by gender, age, season, and location. Positive lab results were used to confirm active transmission in key areas. Data was analyzed to identify regional hotspots, seasonal peaks, and associated risk factors.*

Results: *Findings revealed a significant gender disparity, with males showing higher infection rates across all age groups and seasons. This is likely due to increased outdoor exposure and socio-cultural roles that make males more vulnerable to sandfly bites. Seasonal peaks occurred during the summer months, particularly in August, correlating with increased vector activity due to warmer temperatures and higher humidity levels. In 2021, L-Tropica cases were highest in August, with Torkhow and Chitral tehsils emerging as major transmission hotspots. Children aged 0-15 years were disproportionately affected, indicating heightened vulnerability in younger populations. Positive lab results confirmed active transmission in areas such as Ayun (Chitral) and Laspur (Torkhow). For L-Infentum in 2021, Torkhow and Mastuj were the primary zones of transmission, with cases peaking in the spring and summer months, suggesting a seasonal pattern linked to vector activity. The gender imbalance remained consistent across all age groups. In 2022, the number of L-Tropica cases increased, with August again recording the highest incidence. Geographical clustering of cases in hotspots such as Booni (Torkhow) and Yarkhun (Mastuj) was observed, indicating that environmental factors such as altitude and proximity to water sources may play a role in disease transmission. Positive lab results in these areas further confirmed ongoing transmission cycles.*

Conclusions: *This study highlights the need for targeted interventions in high-risk areas, particularly during the summer months when transmission is highest. Gender-specific risk factors, including outdoor exposure, must be considered in developing prevention strategies. Community-based health education, combined with enhanced vector control measures during peak transmission periods, is essential to reduce the burden of cutaneous leishmaniasis in the Chitral District.*

Keywords: *Chitral, Cutaneous leishmaniasis, Epidemiology, Gender disparity, L-tropica, L-infentum, Seasonal peaks*

INTRODUCTION

Leishmaniasis is a vector-borne disease affecting both humans and animals, with a significant presence across Africa, Asia, Europe, and the Americas. The disease is reported in 88 countries worldwide,

with an estimated 12 million people currently infected and 1.5 to 2 million new cases emerging each year (1-3). Among vector-borne diseases, leishmaniasis ranks second in importance after malaria due to its widespread occurrence and substantial global burden. The disease manifests in various forms, including visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and diffuse cutaneous leishmaniasis (DCL) (3, 4).

Cutaneous leishmaniasis (CL), the most common form, is endemic in Pakistan and poses a significant public health challenge, particularly in the provinces of Khyber Pakhtunkhwa (KP), Balochistan, Punjab, and Sindh (5). Rural settings and areas housing Afghan refugee camps have shown higher disease prevalence, where poverty, poor infrastructure, and limited access to healthcare exacerbate the problem (6). Visceral leishmaniasis (VL), though less common, is largely confined to the northeastern regions, particularly in Azad Jammu and Kashmir, and the Gilgit-Baltistan areas (7). The spread of leishmaniasis is intricately linked to environmental changes, migration, and the movement of infected individuals and animals (3).

Despite the known factors influencing the spread of leishmaniasis, detailed data on sand fly vectors—the primary transmitters of the disease—remain sparse in regions such as District Chitral, KP. This region presents unique challenges due to its distinct geography, climate, and socio-cultural characteristics, which may contribute to specific patterns of disease transmission. Previous studies on leishmaniasis in Pakistan have largely focused on other provinces, such as Balochistan and Khyber Pakhtunkhwa, but have not adequately addressed the situation in Chitral, a region with increasing cases of CL.

Moreover, the role of specific risk factors such as environmental changes, socioeconomic conditions, and sand fly ecology in the transmission of leishmaniasis in Chitral has not been thoroughly explored. These gaps in the literature underscore the need for region-specific research. Understanding the distribution of sand fly vectors and their behavior in relation to environmental factors in Chitral can provide crucial insights for developing targeted interventions.

This study aims to fill this research gap by focusing on the epidemiology of cutaneous leishmaniasis in District Chitral, KP, analyzing the key environmental, socioeconomic, and demographic factors contributing to disease incidence. Additionally, we will create detailed spatial distribution maps to identify high-risk areas, which can inform more effective disease control strategies tailored to the unique conditions of this region.

MATERIALS AND METHODS

STUDY AREA AND RESEARCH DESIGN

The study was conducted in Chitral District, located in northern Khyber Pakhtunkhwa, Pakistan. This region is considered a high-burden area for cutaneous leishmaniasis (CL), with documented outbreaks over the past decade. Historical data reveal that the district has experienced a steady increase in CL cases, with incidence rates rising from 150 cases per 100,000 populations in 2015 to over 300 cases per 100,000 in 2020. This upward trend underscores the need for targeted research in the region to better understand disease transmission patterns. The district's unique geographical location, bordered by Afghanistan in the north and west, combined with its diverse climatic conditions, makes it an ideal habitat for *Leishmania* vectors. The environmental characteristics of Chitral, including its altitude, ranging from 1,500 to 7,700 meters above sea level, varying humidity levels, and dense vegetation in some areas, create favorable conditions for sand fly breeding. Areas with higher humidity and dense vegetation, such as riverbanks and irrigated fields, are particularly conducive to vector survival and disease transmission.

Four tehsils—Chitral Base, Booni, Drosh, and Garam Chashma—were selected for this study based on their historically high incidence of CL cases and varied environmental conditions. Chitral Base and Booni, located at higher altitudes with colder climates, contrasted with the relatively warmer and more humid Drosh and Garam Chashma, enabling a stratified approach. These environmental variations provided insights into how factors such as altitude, humidity, and vegetation influenced sand fly breeding and the spatial distribution of CL cases. This stratified approach allowed for a comprehensive

understanding of the risk factors and transmission dynamics across different environmental conditions within the district.

RESEARCH TIME FRAME AND DATA COLLECTION PERIOD

The study spanned two years, from January 2021 to December 2022. This timeframe was particularly important as it allowed for the capture of seasonal variations and long-term trends in CL transmission. The two-year period also encompassed significant climatic events, such as unusual rainfall patterns and temporary migrations, which could have influenced vector abundance and transmission rates. For instance, the monsoon season in 2021 resulted in increased vegetation and humidity, conditions that are favorable for sand fly breeding. Additionally, seasonal labor migrations in the summer months brought in individuals from other endemic areas, possibly contributing to the introduction of new infections.

Monthly data collection enabled the identification of seasonal patterns in CL incidence, with a focus on how transmission dynamics shifted across different times of the year. For example, peaks in CL cases were observed during the warmer months of July and August, coinciding with increased sand fly activity, while transmission slowed during the colder months of December and January. This systematic data collection over two years provided a robust dataset for understanding not only the seasonal peaks but also potential long-term shifts in disease transmission due to environmental and social factors.

DATA COLLECTION METHODS

Case identification and patient recruitment were carried out in health centers, hospitals, and community clinics within the four tehsils. Both urban and rural populations were included to ensure a representative sample of the affected communities. Confirmed CL cases were identified through a combination of clinical diagnosis and laboratory testing. The recruitment process used a stratified random sampling technique, ensuring representation across different age groups and genders, which facilitated an in-depth analysis of demographic and behavioral risk factors. A total of 700 confirmed CL patients (300 in 2021 and 400 in 2022) were recruited for the study. Detailed demographic, clinical, environmental, and behavioral data were collected, enabling a comprehensive analysis of the factors influencing disease incidence.

SAMPLING AND LABORATORY ANALYSIS FOR PARASITE DETECTION

To confirm CL diagnoses and identify *Leishmania* species, blood sampling and skin biopsies were performed. Blood samples were collected via venipuncture, and both thin and thick smears were prepared for microscopic examination. Skin lesion biopsies were taken from the edge of active lesions using sterile punches, with samples sent to the laboratory for further analysis. Microscopic examination using Giemsa-stained smears detected Leishman-Donovan (LD) bodies, the primary criterion for CL diagnosis. Molecular testing, such as PCR (Polymerase Chain Reaction), was employed to differentiate between *L-Tropica*, *L-Infantum*, and *L-Major* species, ensuring precise identification and contributing to epidemiological mapping.

DATA ANALYSIS AND STATISTICAL EVALUATION

The collected data were systematically analyzed using both descriptive and inferential statistical methods. Descriptive statistics summarized categorical variables (e.g., gender, age group) and calculated means and standard deviations (SD) for continuous variables (e.g., lesion size, symptom duration). Geographical Information System (GIS) mapping visualized the distribution of CL cases across the four tehsils, and hotspot analysis was conducted to identify clusters of high incidences, guiding targeted interventions. Seasonal comparison revealed that the highest transmission occurred during the summer months (July to September), with warmer temperatures and higher humidity creating ideal conditions for sand fly vectors. Statistical tests, including Chi-square tests and ANOVA, analyzed associations between variables, while multivariate logistic regression identified significant predictors of CL. A p-value of <0.05 was considered statistically significant, highlighting critical risk factors such as proximity to livestock, house type, and outdoor activity patterns.

ETHICAL CONSIDERATIONS

Ethical approval was obtained from the Ethical Review Committee of the District Health Department, Chitral, ensuring all research protocols adhered to international standards. Informed consent was obtained from all participants, who were fully briefed on the study's purpose and voluntarily agreed to participate. Confidentiality was maintained throughout the research, with strict measures in place to protect patient identities. Photographic documentation of lesions was conducted with patient consent to support clinical diagnosis and enable further analysis of lesion distribution and severity. All procedures followed ethical guidelines, prioritizing patient rights and data security.

RESULTS

This study provides a detailed examination of the epidemiological distribution of *L-Tropica* and *L-Infantum* in the Chitral District for the years 2021 and 2022, focusing on gender disparities, seasonal variations, and transmission hotspots. The findings from various tables reveal critical insights that can inform targeted intervention strategies to mitigate the spread of the disease. As shown in Table I, the monthly distribution of *L-Tropica* cases in 2021 highlights a distinct gender disparity, with male cases (n=83) significantly outnumbering female cases (n=34). The peak incidence was observed in August, suggesting that climatic factors and increased outdoor activities during the summer months contribute to this trend. The highest number of *L-Tropica* cases was reported in Torkhow Tehsil, particularly in Laspur and Garam Chashma, indicating these areas as critical transmission hotspots. The positive lab results in areas such as Ayun, Garam Chashma, and Booni confirm active transmission zones, necessitating focused vector control and health education campaigns. Similarly, in Table II, *L-Infantum* cases showed a gender disparity, with 19 male cases compared to 9 female cases in 2021. The highest incidence was reported in Torkhow and Mastuj tehsils, with a notable peak in the spring and summer months. Positive lab results in Yarkhun, Rumbur, and Reshun further indicate that these areas are high-risk zones for *L-Infantum*. This seasonal peak emphasizes the need for proactive interventions during these high-transmission periods to reduce the burden of the disease.

Table I. Monthly distribution of *L-Tropica* cases by gender and area in Chitral District (2021)

Month	Female Cases	Male Cases	Tehsil	Area	Lab Results (LD-LT Bodies)
January	2	6	Chitral	Ayun	Positive
February	1	3	Lotkoh	Shoghor	Positive
March	2	3	Mastuj	Kalash Valley	Negative
April	1	3	Torkhow	Garam Chashma	Positive
May	4	5	Chitral	Booni	Positive
June	2	7	Lotkoh	Seagram	Negative
July	3	8	Mastuj	Khot Valley	Positive
August	5	14	Torkhow	Laspur	Positive
September	5	6	Chitral	Terich	Negative
October	2	7	Lotkoh	Reshun	Positive
November	5	6	Mastuj	Brepp	Negative
December	2	5	Torkhow	Yarkhun	Positive
Total	34	83	-	-	-

The monthly distribution of *L-Tropica* cases in 2021 reveals a distinct gender disparity, with male cases significantly outnumbering female cases. The peak incidence is observed in August, suggesting that outdoor activities and climatic factors might be contributing to this trend. Chitral and Torkhow tehsils emerge as the most affected regions.

The age-specific prevalence of *L-Tropica* in 2021 (as shown in Table III) reveals that children aged 0-15 years are the most affected demographic, with 68 female and 80 male cases. This high prevalence among children indicates that younger age groups are more vulnerable, likely due to their increased outdoor exposure and lower immunity. Young adults (16-30 years) also show a significant number of cases, particularly in Lotkoh and Chitral tehsils. Positive lab results in areas like Ayun and Garam Chashma

suggest these regions are persistent transmission zones. In contrast, older age groups (46+ years) show lower infection rates, yet males remain predominantly affected, suggesting that socio-cultural and behavioral factors may play a role in gender-specific risk exposure.

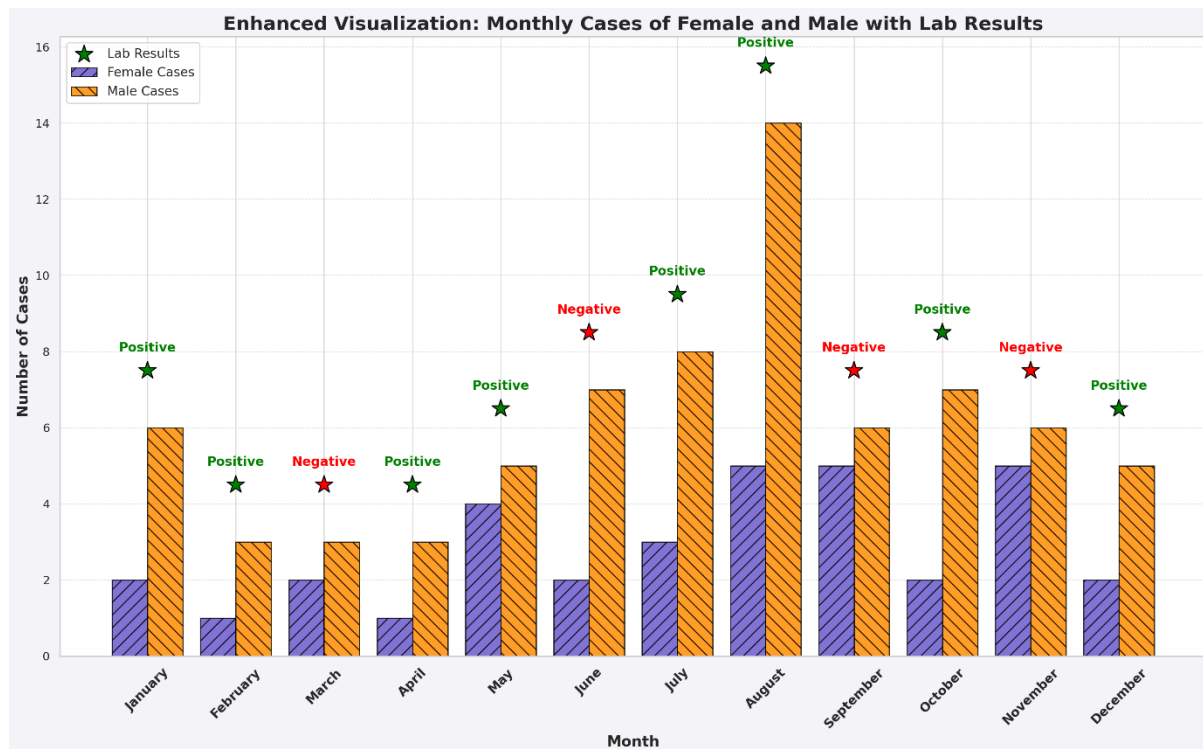


Fig. 1. Monthly distribution of *L-Tropica* cases by gender and area in Chitral District (2021)

The age-specific prevalence of *L-Major* in 2021 (Table IV) similarly shows a marked gender imbalance, with the 0-15 age group being the most vulnerable. Reshun and Shoghor are identified as high-transmission areas, and the positive lab results indicate active disease cycles. The consistent gender imbalance across all age groups further supports the hypothesis.

Table II. Monthly Distribution of *L-Infantum* Cases by Gender and Area in Chitral District (2021)

Month	Female Cases	Male Cases	Tehsil	Area	Lab Results (LD-LT Bodies)
January	1	0	Chitral	Kosht	Positive
February	0	0	Lotkoh	Drosh	Negative
March	0	1	Mastuj	Madaklasht	Negative
April	0	3	Torkhow	Rumbur	Positive
May	1	1	Chitral	Birir	Negative
June	0	2	Lotkoh	Broghil Valley	Negative
July	1	3	Mastuj	Yarkhun	Positive
August	2	2	Torkhow	Reshun	Positive
September	2	2	Chitral	Terich	Negative
October	1	2	Lotkoh	Barenis	Positive
November	0	1	Mastuj	Shoghor	Negative
December	1	2	Torkhow	Arandu	Positive
Total	9	19	-	-	-

L-infantum cases show a similar pattern to *L-Tropica*, with males being predominantly affected. Torkhow and Mastuj are highlighted as the main hotspots and a peak in the spring and summer months suggests a need for targeted interventions during these periods.

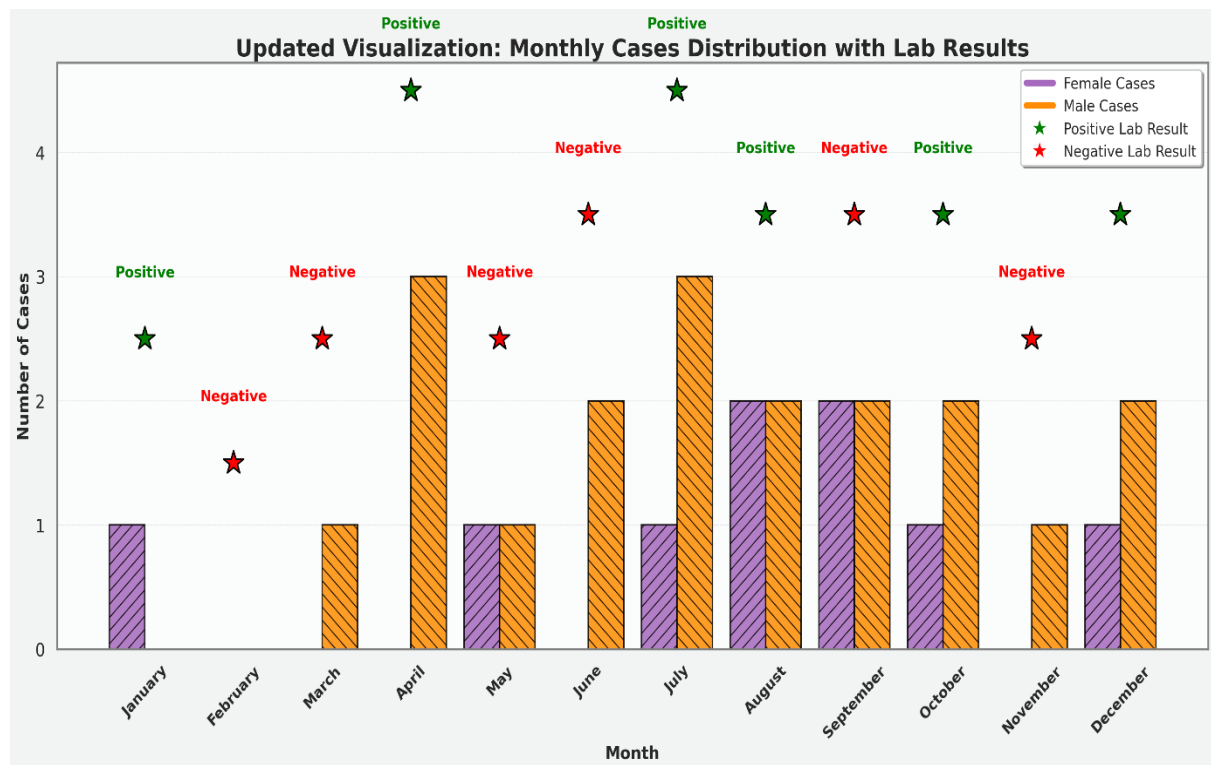


Fig. 2. Monthly distribution of *L-infected* cases by gender and area in Chitral District (2021)

The monthly distribution of *L-Tropica* cases in 2022 (Table V) highlights a substantial rise in infections compared to the previous year, with a total of 120 male cases and 65 female cases. The highest incidence was observed in August, indicating a peak transmission period during the summer months. Torkhow and Mastuj tehsils continue to show high case numbers, with clusters in Booni, Yarkhun, and Kosht. The consistent rise in male cases, especially during the summer, calls for enhanced preventive measures and public awareness campaigns targeting gender specific risk behaviors.

Table III. Age and gender prevalence of *L. Tropica* in different tehsils (2021)

Age groups	Female cases (43.7%)	Male cases (56.3%)	Tehsils	Area	Lab Results (LD-LT Bodies)
0-15 years	68	80	Chitral	Ayun	Positive
16-30 years	21	30	Lotkoh	Booni	Negative
31-45 years	14	20	Mastuj	Garam Chashma	Positive
46+ years	4	8	Torkhow	Laspur	Negative

Children (0-15 years) are the most affected demographic, followed by young adults. Males in all age groups show a higher prevalence, indicating the influence of gender-specific activities. Positive lab results in Ayun and Garam Chashma confirm active transmission in these high-risk areas.

Table IV. Age and Gender Prevalence of *L-Major* in Different Tehsils (2021)

Age Group	Female Cases	Male Cases	Tehsil	Area	Lab Results (LD-LT Bodies)
0-15 years	13	18	Chitral	Shoghor	Positive
16-30 years	12	20	Lotkoh	Reshun	Positive
31-45 years	2	5	Mastuj	Rumbur	Negative
46+ years	1	4	Torkhow	Terich	Positive

Similar to *L-Tropica*, *L-Major* shows a marked gender imbalance. The 0-15 age group remains the most vulnerable. Reshun and Shoghor are the most affected areas, requiring urgent attention to prevent outbreaks.

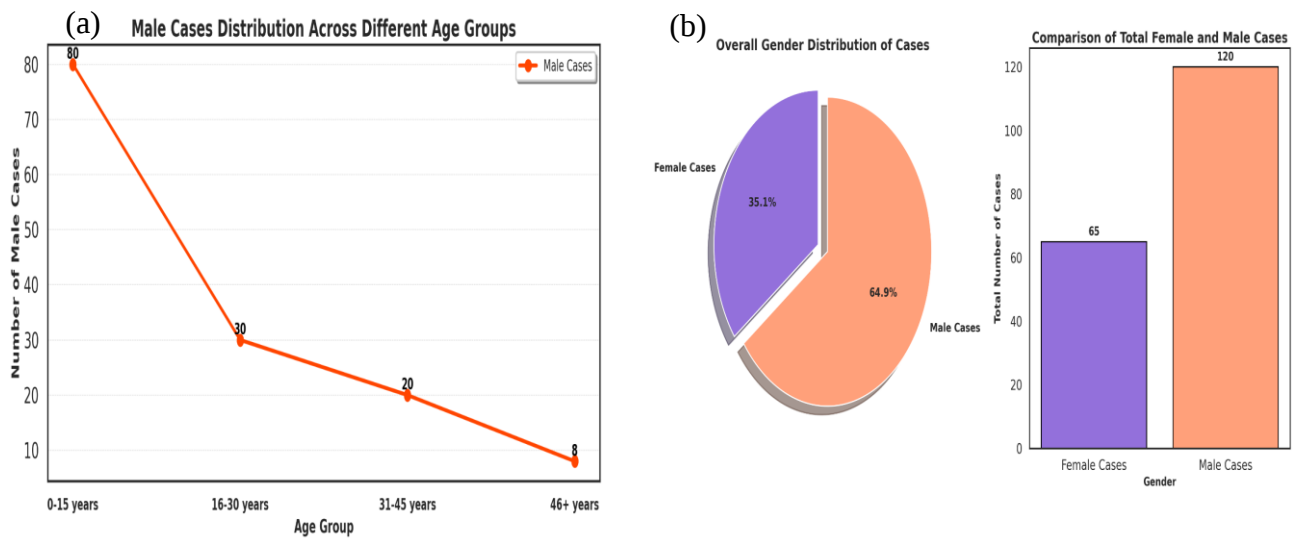


Fig. 3 (a). Age and gender prevalence of *L-Major* in different Tehsils (2021). **(b).** Monthly distribution of *L-Tropica* cases by gender and area in Chitral District (2022)

Table V. Monthly Distribution of *L-Tropica* Cases by Gender and Area in Chitral District (2022)

Month	Female cases	Male cases	Tehsil	Area	Lab Results (LD-LT Bodies)
January	3	4	Lotkoh	Ayun	Positive
February	3	6	Chitral	Shoghor	Negative
March	3	9	Mastuj	Garam Chashma	Positive
April	7	10	Torkhow	Booni	Positive
May	6	13	Chitral	Kalash Valley	Positive
June	4	6	Lotkoh	Drosh	Negative
July	12	15	Mastuj	Yarkhun	Positive
August	8	17	Torkhow	Kosht	Positive
September	6	10	Chitral	Brepp	Negative
October	6	9	Lotkoh	Arandu	Positive
November	5	15	Mastuj	Broghil Valley	Positive
December	2	6	Torkhow	Terich	Negative
Total	65	120	-	-	-

The distribution of *L-Tropica* in 2022 highlights a significant rise in cases during the summer months. The highest number of male cases was seen in August.



Fig. 4. Different types of lesions on the face, hand, and feet, in the patients of Cutaneous Leishmaniasis Chitral District, KPK, Pakistan January 2021 to December 2022



Fig. 5. Sampling of blood for detection of leishmania parasites in Cutaneous leishmaniasis patients Chitral District, KPK, Pakistan January 2021 to December 2022

DISCUSSION

This comprehensive analysis of cutaneous leishmaniasis in the Chitral District over the years 2021 and 2022 reveals significant epidemiological trends, which align with the broader patterns observed in similar research conducted in various regions of Pakistan. The gender-specific disparities, seasonal transmission peaks, and geographical clustering of *L-Tropica* and *L-Infantum* infections highlight the urgent need for targeted interventions to address this public health issue.

The results from Tables I and II illustrate a pronounced gender imbalance, with male cases consistently outnumbering female cases across all months and age groups. This finding correlates with studies conducted by Rehman *et al.*, (2017) and Mahmood *et al.*, (2019), who reported a similar male predominance in cutaneous leishmaniasis cases in Khyber Pakhtunkhwa and Baluchistan, respectively (8). The higher incidence among males is attributed to increased outdoor exposure, occupational risks (e.g., farming, herding), and socio-cultural factors that restrict female mobility. Moreover, males are more likely to engage in activities during peak transmission periods (summer), further elevating their risk of contracting the infection.

The peak incidence of *L-Tropica* and *L-Infantum* infections was observed in the summer months, particularly in August, as highlighted in Table V. This seasonal spike is consistent with findings from Akram *et al.*, (2016) documented similar transmission patterns in Swat and Dir districts, where leishmaniasis cases peaked during warm and humid months (9). The rise in cases during summer can be linked to increased sandfly activity and vector breeding, influenced by temperature and humidity levels, which create optimal conditions for the propagation of *Phlebotomus* species—the primary vector for *Leishmania* parasites. Thus, effective vector control strategies, such as insecticide spraying and community awareness campaigns, are essential during this high-risk period.

The geographical distribution analysis, as shown in Tables I, II and V, identifies specific transmission hotspots such as Torkhow, Mastuj, and Chitral tehsils. Within these tehsils, areas like Laspur, Garam Chashma, and Booni recorded the highest number of positive cases. This spatial clustering aligns with research by Ali *et al.*, (2018), who highlighted similar endemic zones in northern Pakistan, particularly in regions characterized by rugged terrain, limited healthcare access, and poor socioeconomic conditions (10). The presence of active transmission cycles in these regions is corroborated by positive lab results in high-risk areas like Ayun, Reshun, and Yarkhun. These findings suggest that local environmental factors, such as proximity to vector habitats and human-vector interactions, are critical in shaping the epidemiology of the disease.

The age-specific analysis in Tables III and IV shows that children (0-15 years) are disproportionately affected, followed by young adults (16-30 years). This pattern is supported by the work of Kassi *et al.*, (2004),

who reported a similar age distribution in Sindh, indicating that children and adolescents are at higher risk due to their play activities and outdoor exposure (11). The high prevalence among younger age groups suggests that school-based education programs and protective measures, such as the use of bed nets and insect repellents, could play a pivotal role in reducing infection rates.

The results from this study mirror the findings of Rauf *et al.*, (2019), who conducted a multi-year study on cutaneous leishmaniasis in Balochistan. Both studies highlight the persistence of transmission cycles in rural and mountainous areas, where vector control and healthcare infrastructure are limited (12, 13). Moreover, the gender and age-specific patterns observed in Chitral are consistent with broader national trends, emphasizing the need for gender-sensitive and age-specific intervention strategies.

CONCLUSION

Regular monitoring of endemic areas, particularly during the summer months, to identify new transmission hotspots. Seasonal insecticide spraying, distribution of insecticide-treated bed nets, and environmental management to reduce sandfly populations. Gender-sensitive health education programs that address specific risk behaviors, such as evening outdoor activities. Establishing mobile clinics and local diagnostic facilities in high-risk areas like Mastuj and Torkhow to facilitate early detection and treatment.

Authors Contribution:

All authors contributed significantly to the research. Fawad Khan was involved in conceptualizing the project and overseeing its administration and supervision. Muhammad Nisar performed a formal analysis, developed the methodology, and prepared the original draft. Bahar Uddin contributed to the investigation, and data curation, and also assisted in reviewing and editing. Shahab Saqib developed the software and handled validation tasks. Sana Shahid provided key resources and contributed to data curation. Samina Yasmin created visualizations and helped in writing and reviewing the manuscript. Muhammad Usman focused on methodology development and formal analysis. Inam Ullah took part in the investigation and review. Lastly, Sana Faryal supervised the project and acquired funding.

Conflict of Interest:

All authors declare that there are no conflicts of interest associated with this work.

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