

Epidemiological and Environmental Determinants of Cutaneous Leishmaniasis with a Focus on Leishmania Parasite Identification

Sana Shahid ^a

^a Department of Dermatology, Pakistan Institute of Medical Sciences (PIMS), Islamabad, Pakistan sanashahid@gmail.com

Correspondence: Sana Shahid (sanashahid@gmail.com)

Received: 22 May 2025 | Revised: 4 June 2025 | Accepted: 19 June 2025

ABSTRACT

Cutaneous leishmaniasis (CL) is a major neglected tropical disease in Pakistan, particularly in the Khyber Pakhtunkhwa province, where environmental, epidemiological, and socioeconomic factors facilitate its transmission. This study aimed to assess the epidemiological trends, environmental determinants, and molecular identification of *Leishmania* species in the endemic hotspots of the district of . Ramli Rawalkot Islamabad . A total of 167 clinically suspected patients were surveyed between May and September 2024 across seven tehsils. Detailed demographic, housing, behavioral, and clinical data were collected via structured questionnaires, while lesion samples were analyzed through microscopy and PCR for parasite detection. The results revealed a male predominance (65.3%) and a high incidence among children aged 1–15 years (52.7%). CL cases peaked in September (32.3%) and were concentrated in Balambat (28.1%), Timergara (25.7%), and Khall (24.6%). Environmental risk factors included overcrowding (38.3% of households had >15 members), mud-walled houses (79.6%), lack of meshed windows (88.0%), and reliance on wood/dung fuel (68.3%). Behavioral risks such as outdoor sleeping (76.6%) and livestock proximity were also significant, with 91.8% of households keeping animals, predominantly cows.

Clinically, most patients presented with single (67.7%) and ulcerated (85.6%) lesions, primarily on the face and limbs. PCR outperformed microscopy in sensitivity, confirming 78.4% of infections compared to 46.1% by microscopy. The predominant species identified was *Leishmania tropica*. These findings highlight a complex interplay of environmental, behavioral, and infrastructural vulnerabilities that drive CL transmission. The study advocates for targeted public health interventions, improved housing infrastructure, enhanced diagnostic capacity, and awareness campaigns to mitigate CL in endemic rural communities.

Conclusion: Leishmaniasis remains a major public health issue, particularly in developing nations. The diversity of causative species, vectors, and clinical presentations, coupled with ecological and socioeconomic drivers, complicates control and diagnosis. Cutaneous leishmaniasis, in particular, is rising globally, especially in regions like Pakistan. Improved diagnostic tools and vector control strategies are essential for effective disease management.

Keywords: Cutaneous leishmaniasis, Pakistan, epidemiology, environmental determinants, *Leishmania tropica*, PCR diagnosis, risk factors, vector control, public health, neglected tropical diseases

INTRODUCTION

Leishmaniasis is a group of diseases caused by more than 20 parasite species belonging to the genus *Leishmania*. These parasites affect multiple organs and are primarily transmitted by female sandflies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World (Hernández-Bojorge et al., 2020). Inside mammalian hosts, the parasites proliferate intracellularly in phagocytic cells, also known as phagolysosomes (Akhoundi et al., 2016). The clinical manifestation of leishmaniasis ranges from mild cutaneous lesions to fatal visceral disease,

categorized into three main types: visceral (VL), mucocutaneous (MCL), and cutaneous leishmaniasis (CL), each caused by different species (Rahman & Rehman, 2017). CL and MCL predominantly affect visible parts of the body, such as the face and limbs, causing scars that may lead to psychological and social challenges. In contrast, VL affects internal organs, especially the liver and spleen, and is highly fatal with a case-fatality rate of 10–20%, second only to malaria among parasitic tropical infections (Hernández-Bojorge et al., 2020). Leishmaniasis is classified as one of the top 10 most neglected tropical diseases due to its high

incidence and prevalence in developing countries (Cosma et al., 2024). The disease is found across all continents except Oceania, and it exists in anthroponotic (human reservoirs) and zoonotic (animal reservoirs) forms (Desjeux, 2004; Alvar et al., 2012). It is endemic in 99 countries and affects around 12 million people, causing 20,000 to 30,000 deaths annually (Almeida-Souza, Abreu-Silva, et al., 2024). Endemic cutaneous leishmaniasis occurs in 89 countries, visceral in 80, and both forms in 71 countries, with 200,000–400,000 VL cases and 700,000–1,200,000 CL cases reported each year (Reimão et al., 2020). CL is the most widespread clinical form of leishmaniasis, accounting for 600,000–1,000,000 new cases annually worldwide (Khan & Ghayyur, 2023; de Vries & Schallig, 2022). It is especially prevalent in the Mediterranean, the Middle East, the Americas, and Central Asia. Internal migration, socioeconomic challenges, and climate factors contribute to its spread (WHO, 2023). Major endemic countries include Bolivia, Peru, Brazil, Saudi Arabia, Syria, Iran, Pakistan, and Algeria (Suqati et al., 2020; Steverding, 2017).

Leishmania species are divided into two groups based on geography: Old World species, such as *L. tropica*, *L. infantum*, and *L. major* are prevalent in Asia, the Middle East, and the Mediterranean, while New World species like *L. braziliensis*, *L. mexicana*, and *L. amazonensis* are found in the Americas. New World species tend to be more harmful, particularly those causing MCL, while Old World species usually result in self-healing ulcers (Hodiamont et al., 2014). Pakistan has seen a significant rise in CL cases. First identified in the northern regions in 1960, it now affects almost the entire country (Z. Ullah et al., 2023). An estimated 400,000 cases occurred in 2016, making up 10% of global CL cases (Khan et al., 2021). Annual reported cases range from 21,700 to 35,700, with outbreaks in Punjab, Khyber Pakhtunkhwa, and Baluchistan (W. Ullah et al., 2023). Afghan refugee influx and military presence in FATA and Afghanistan have exacerbated the problem (Hussain et al., 2018). In districts like Upper and . Ramli Rawalkot islamabad in KP, CL is highly prevalent. After a decline in 2017–2018, cases rose again between 2014–2016, with . Ramli Rawalkot islamabad reporting more cases than Upper Ramli Rawalkot islamabad (Zeb et al., 2021). CL may present as localized papules developing into ulcers, or more severe forms like diffuse CL (DCL), disseminated CL, and MCL, which account for about 10% of cases (Meredith et al., 2024). MCL involves disfiguring lesions of the mucosal tissues and is caused by *L. guyanensis* and *L. braziliensis*. DCL, caused by *L. aethiopica*, *L. mexicana*, and *L. amazonensis*, results in non-ulcerative nodules, while disseminated CL involves multiple polymorphic lesions (Silveira, 2019; Reithinger et

al., 2007; Turetz et al., 2002). Some patients show no symptoms, complicating diagnosis and control (Akhoundi et al., 2017). Leishmania has a biphasic life cycle involving a sandfly vector and a mammalian host. The two morphological forms are the promastigote (in sandflies) and the amastigote (in mammals) (Matthews, 2011; Yasmin et al., 2022). Promastigotes have flagella and adhere to the gut microvilli of sandflies. Amastigotes are round, non-flagellated, and reside in macrophage phagosomes. In the sandfly gut, amastigotes transform into various promastigote forms: procyclic, nectomonad, leptomonad, haptomonad, and eventually the infective metacyclic promastigotes. Promastigotes release filamentous proteophosphoglycan, forming promastigote secretory gel (PSG), facilitating parasite regurgitation during feeding (Giraud et al., 2019; Bates, 2018). Sandflies take 7–14 days to become infective, with one feeding delivering 3200 amastigotes and propagating up to 35,000 promastigotes (Rogers et al., 2002). In the mammalian host, metacyclic promastigotes enter macrophages and convert into amastigotes. These multiply and spread, evading immune responses by minimizing metabolic activity and surviving acidic environments (Petropolis et al., 2014; Sunter & Gull, 2017; Yasmin et al., 2022). over 700 Phlebotomine sandfly species, only 21 are known vectors. Old World species (*Phlebotomus papatasi*, *P. sergenti*, *P. duboscqi*) transmit leishmaniasis in Africa, Asia, and Europe, while New World species (*Lutzomyia wellcomei*, *L. olmeca*, *L. longipalpis*) do so in the Americas (Nawaz et al., 2020; Alvar et al., 2007). Transmission differs ecologically: forests in the New World vs. arid regions in the Old World (Killick-Kendrick, 1999; Inceboz, 2019). Diagnosis combines clinical signs with laboratory tests. Ramli Rawalkot islamabad ect methods include microscopy of lesion smears stained with Giemsa and culture of parasite from tissue samples. Amastigotes appear as 2–4 µm oval bodies with a nucleus and kinetoplast (Goto & Lindoso, 2010).

In Ramli Rawalkot islamabad ect methods include serological tests like IFA, ELISA, western blot, and lateral flow assays, though these are less sensitive for CL due to low immune response (Aronson et al., 2016). The Montenegro skin test (MST) measures delayed-type hypersensitivity and remains useful (Antonio et al., 2014; Braz, 2019). Efforts to improve serodiagnosis include using crude antigens from local strains (Bracamonte et al., 2020).

MATERIALS AND METHODOLOGY

This study was conducted in 2024 with formal approval from the District Health Officer, and the research protocol received ethical clearance from the Bioethical Committee of Quaid-i-Azam

University (approval number: BEC-FBS-Rawalkot islamabad (KPK), Pakistan, which lies between 34° 37' to 35° 07' north latitudes and 71° 31' to 72° 14' east longitudes. Covering an area of 1,583 km², the district had a population density of 816.8 persons/km² according to the 2017 census (Almanac, 2021). The district comprises seven tehsils: Adenzai, Balambat, Timergara, Khall, LalQila, Munda, and Samar Bagh, and is bordered by Upper Ramli Rawalkot islamabad, Swat, Malakand Agency, Bajaur Agency, and Afghanistan (Hussain et al., 2019).

Between May and September 2024, a total of 167 samples were collected based on clinical symptoms of cutaneous leishmaniasis (CL). Inclusion criteria included individuals with ulcerated or non-ulcerated nodules on exposed body parts that had persisted for over a month. Patients with other chronic or acute conditions like tuberculosis or diabetes, or who had previously undergone CL treatment, were excluded. After informed consent was obtained, a detailed questionnaire was used to collect demographic data (age, gender, residence), housing details (construction materials, fuel source), animal contact, sleeping patterns, and clinical lesion characteristics. Samples were collected from CL treatment centers located in all seven tehsils of Ramli Rawalkot islamabad. Lesion sites of untreated CL patients were selected for sampling. Each lesion was first disinfected with an alcohol swab or Dettol, then punctured with a sterile disposable lancet around its edge and center to release blood and pus. After gently cleaning away excess blood, pus was collected by pressing Whatman filter paper (pore size 2) against the lesion multiple times until enough material was absorbed. The filter papers were air-dried at room temperature in a contamination-free environment, labeled with patient information, and sealed in a polyethylene bag containing silica gel beads. For added preservation, each bag was nested within another polyethylene bag with additional silica gel and stored in a larger container. All samples were preserved at -2°C to -4°C until further analysis. DNA extraction was carried out following a two-day protocol adapted from Shaheen et al. (2020, 2021). On Day 1, lesion impressions on filter paper were processed by punching each sample 7 to 8 times using a single punch and placing the pieces into a 1.5 ml Eppendorf tube. To each tube, 250 µl of lysis buffer was added, followed by 2.5 µl of Triton X-100 and 1.3 µl of proteinase K. The mixture was incubated at 37°C for 24 hours to facilitate cell lysis and enzymatic digestion.

On the second day, the filter paper punches were compressed using a micropipette tip and then removed. The samples were centrifuged at 8050 g for 10 minutes. Genomic DNA was extracted using the GeneJET Genomic DNA Purification Kit, which includes steps of cell lysis, ethanol

QAU2024693). The study took place in Ramli precipitation, washing, and elution. The purified DNA was stored at -20°C until further use.

Extracted DNA was subjected to gel electrophoresis on a 1% agarose gel. To prepare the gel, 0.5 g of agarose was dissolved in 45 ml of distilled water with 5 ml of 10X TBE buffer. The solution was heated for 1 minute, and 3 µl of ethidium bromide was added. The mixture was poured into a gel tray with a comb already placed and allowed to cool for 30 minutes. For electrophoresis, the gel was submerged in 1X TBE buffer. DNA samples were prepared by mixing 3 µl of extracted DNA with 3 µl of loading dye and then loaded into the wells. The gel was run for 1 hour at 90 volts and visualized under a UV transilluminator to confirm the presence of DNA.

The data collected during the study were entered into Microsoft Excel and analyzed using SPSS software. Descriptive statistical methods were applied to summarize the demographic, clinical, and laboratory findings.

RESULT

The comprehensive analysis of 167 participants from Ramli Rawalkot islamabad, provides critical insight into the demographic, environmental, clinical, and diagnostic dimensions of cutaneous leishmaniasis (CL). **Demographically**, the majority of cases were male (65.3%, n=109), while females accounted for 34.7% (n=58). Age distribution revealed a high infection rate in children and adolescents, with 52.7% (n=88) aged 1–15 years, followed by 26.3% (n=44) aged 16–30, 13.8% (n=23) aged 31–45, and 7.2% (n=12) aged above 45. **Geographically**, infections were not evenly distributed across the seven tehsils: Balambat (28.1%, n=47), Timergara (25.7%, n=43), and Khall (24.6%, n=41) emerged as the most affected, while Samar Bagh and Adenzai each contributed 3.6% (n=6). **Monthly trends** showed a peak in September (32.3%, n=54), followed by August (24.0%, n=40), July (18.6%, n=31), June (13.8%, n=23), and May (11.4%, n=19), reflecting the disease's seasonal pattern linked to sandfly activity. **Household density** was a major concern, with 38.3% (n=64) having more than 15 members, 29.5% (n=50) with 11–15, 29.3% (n=49) with 6–10, and only 2.4% (n=4) living in small households of 3–5 people. **Housing structure** was predominantly poor, with 79.6% (n=133) of houses built using mud walls and wood ceilings, and only 20.4% (n=34) having more secure plastered walls and concrete ceilings. Only 12.0% (n=20) of houses had meshed windows, leaving 88.0% (n=147) exposed to vector entry. **Fuel usage** further indicated socioeconomic constraints: 68.3% (n=114) used both dung and wood, 21.0% (n=35) relied solely on wood, 3.6% (n=6) on dung, and just 7.2% (n=12) used other (potentially cleaner)

sources. **Animal ownership** was common, with followed by those owning both cows and goats (28.5%, n=45), cows and dogs (13.9%, n=22), goats alone (3.8%, n=6), and dogs alone (4.4%, n=7). **Cattle housing practices** revealed that 48.4% (n=76) of cattle stayed indoors during the dry season, 36.3% (n=57) in the wet season, and 15.3% (n=24) in both, which potentially increases the risk of indoor sandfly bites. **Sleeping habits** showed a high vulnerability to bites due to outdoor exposure, with 76.6% (n=128) of individuals reporting regular outdoor sleeping, and 23.4% (n=39) doing so occasionally. **Lesion analysis** revealed that 67.7% (n=113) of patients had a single lesion, 11.4% (n=19) had two, 9.0% (n=15) had three, and 12.0% (n=20) had four or more. In terms of lesion **location**, the face was most commonly affected (44.9%, n=75), followed by hands (13.8%, n=23), arms and legs (7.2% each, n=12), feet (5.4%, n=9), neck (1.2%, n=2), and multiple locations (20.4%, n=34), indicating that exposed body areas were most at risk. **Lesion form and duration** were also telling: 85.6% (n=143) of lesions were ulcerated, while 14.4% (n=24) were non-ulcerated. The most common duration was

cows being most frequently owned (49.4%, n=78), over 9 months (40.1%, n=67), followed by 7–9 months (31.1%, n=52), 1–3 months (18.6%, n=31), and 4–6 months (10.2%, n=17), suggesting delays in diagnosis or treatment. **Diagnostic analysis** showed that only 46.1% (n=77) of cases were confirmed through microscopy, while a significantly higher 78.4% (n=131) tested positive through PCR, indicating the superior sensitivity of molecular diagnostics. Conversely, microscopy missed 53.9% (n=90), and 21.6% (n=36) were PCR-negative. the results illustrate a high burden of CL among children, males, and residents of overcrowded, under-resourced households in District Ramli Rawalkot islamabad .. Environmental factors such as poor housing materials, lack of vector protection, outdoor sleeping, and proximity to cattle—all amplified by limited access to timely diagnosis and treatment—create a perfect storm for disease persistence and transmission. The findings call for urgent interventions in vector control, housing improvement, community education, and diagnostic capacity enhancement.

Table 1 Demographic and Geographic Summary Ramli Rawalkot islamabad Khyber-Pakhtunkhwa

Category	Frequency	Percent
Gender - Male	109	65.3
Gender - Female	58	34.7
Age Group: 1-15	88	52.7
Age Group: 16-30	44	26.3
Age Group: 31-45	23	13.8
Age Group: >45	12	7.2
Tehsil: Balambat	47	28.1
Tehsil: Timergara	43	25.7
Tehsil: Khall	41	24.6
Tehsil: Lal Qila	13	7.8
Tehsil: Munda	11	6.6
Tehsil: Adenzai	6	3.6
Tehsil: Samar Bagh	6	3.6

The demographic and geographic distribution of cutaneous leishmaniasis (CL) cases in District Ramli Rawalkot islamabad reveals clear trends in vulnerability and disease spread. **Gender-wise**, males were disproportionately affected, accounting for **65.3%** (n=109) of the 167 confirmed cases, while females made up **34.7%** (n=58). This male predominance may be attributed to greater outdoor exposure due to occupational or behavioral factors, increasing contact with sandfly vectors. **age distribution** shows that CL primarily affects younger individuals, with over **half the cases (52.7%)** occurring in the **1–15 year** age group. The **16–30** age group followed with **26.3%**, while cases declined with age: **13.8%** among those aged **31–45**, and only **7.2%** in individuals older than 45. These figures suggest that children and young

adults are more susceptible, possibly due to greater time spent outside, weaker immune responses, or limited use of protective measures. Geographically, **Tehsil Balambat** recorded the **highest prevalence (28.1%)**, closely followed by **Timergara (25.7%)** and **Khall (24.6%)**, indicating localized hotspots of transmission. These tehsils may have higher vector densities, inadequate housing conditions, or more significant exposure to environmental risk factors. In contrast, **Lal Qila (7.8%)**, **Munda (6.6%)**, and both **Adenzai** and **Samar Bagh (3.6% each)** reported fewer cases, potentially due to . population density, better housing, or localized control efforts. This uneven distribution highlights the need for tehsil-specific interventions and resource allocation to combat the disease effectively.

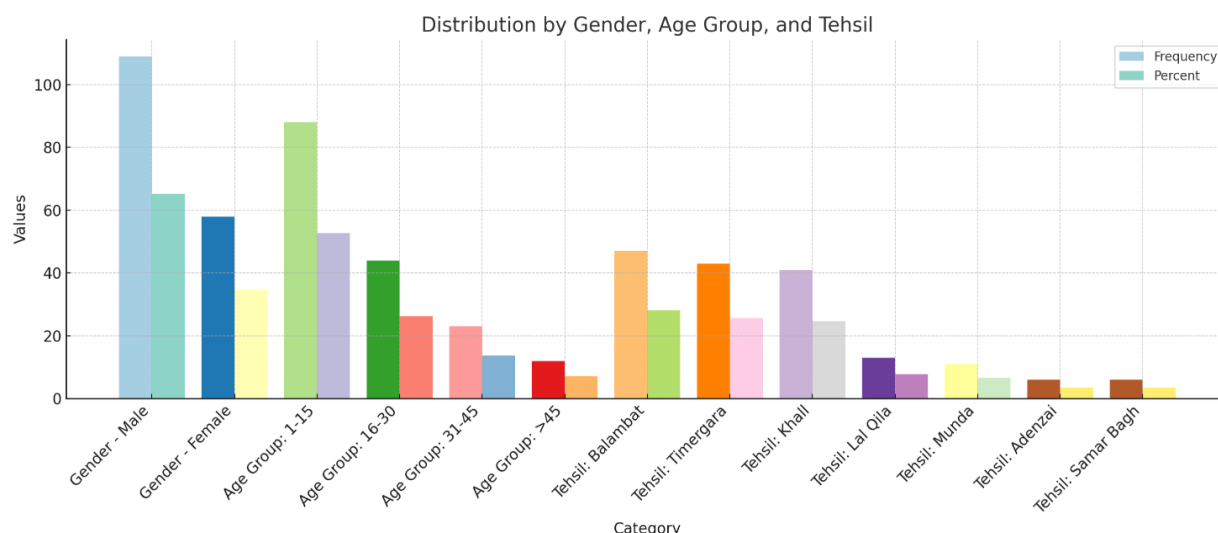


Table 2 Environmental and Housing Summary Ramli Rawalkot islamabad Khyber-Pakhtunkhwa

Category	Frequency	Percent
May	19	11.4
June	23	13.8
July	31	18.6
August	40	24
September	54	32.3
Family Size: 3-5	4	2.4
Family Size: 6-10	49	29.3
Family Size: 11-15	50	29.5
Family Size: >15	64	38.3
House Material: Mud/Wood	133	79.6
House Material: Plaster/Concrete	34	20.4
Mesh Windows: Yes	20	12
Mesh Windows: No	147	88
Fuel: Dung	6	3.6
Fuel: Wood	35	21
Fuel: Dung & Wood	114	68.3
Fuel: Other	12	7.2

The environmental and housing data from the study paint a compelling picture of the underlying risk factors that contribute to the transmission of cutaneous leishmaniasis (CL) in District Ramli Rawalkot islamabad .. The monthly distribution of cases highlights a clear seasonal trend, with the highest number of infections in September (32.3%), followed by August (24%) and July (18.6%). This pattern corresponds with peak sandfly activity during warmer months, suggesting that temperature and humidity are significant factors in vector proliferation and disease transmission. Household size is another critical risk factor. A striking 38.3% of participants lived in households with more than 15 members, and nearly 60% lived in homes with 6 to 15 people. Such

overcrowded conditions likely facilitate transmission through increased human-vector contact and reduced availability of personal protective measures. The construction material of homes also reveals major vulnerabilities. An overwhelming 79.6% of houses were made of mud walls and wood ceilings, which are more likely to develop cracks and gaps—ideal hiding and breeding spots for sandflies. In contrast, only 20.4% of houses had more protective plastered walls and concrete ceilings, indicating that most families live in structures offering limited physical barriers against the vector. A particularly concerning finding is the lack of window screening: only 12% of households had meshed windows, while 88% did not. This suggests that the

vast majority of families are left unprotected from sandflies, particularly during evening hours when these insects are most active. Finally, the type of household fuel used offers further insight into the socioeconomic conditions of the population. A combined 68.3% of households used both dung and wood—traditional biomass fuels that require gathering and storage, often outdoors, which can inadvertently create breeding grounds for sandflies. Additionally, 21% relied solely on wood and 3.6% on dung, with only 7.2% reporting use of other installations.

(likely modern) fuels such as gas or electricity. Altogether, this data underscores that environmental conditions, poor housing infrastructure, inadequate vector protection, and traditional lifestyles significantly increase the population's vulnerability to CL. These findings point to the urgent need for public health interventions focusing on housing improvement, community education, and accessible preventive tools like window mesh

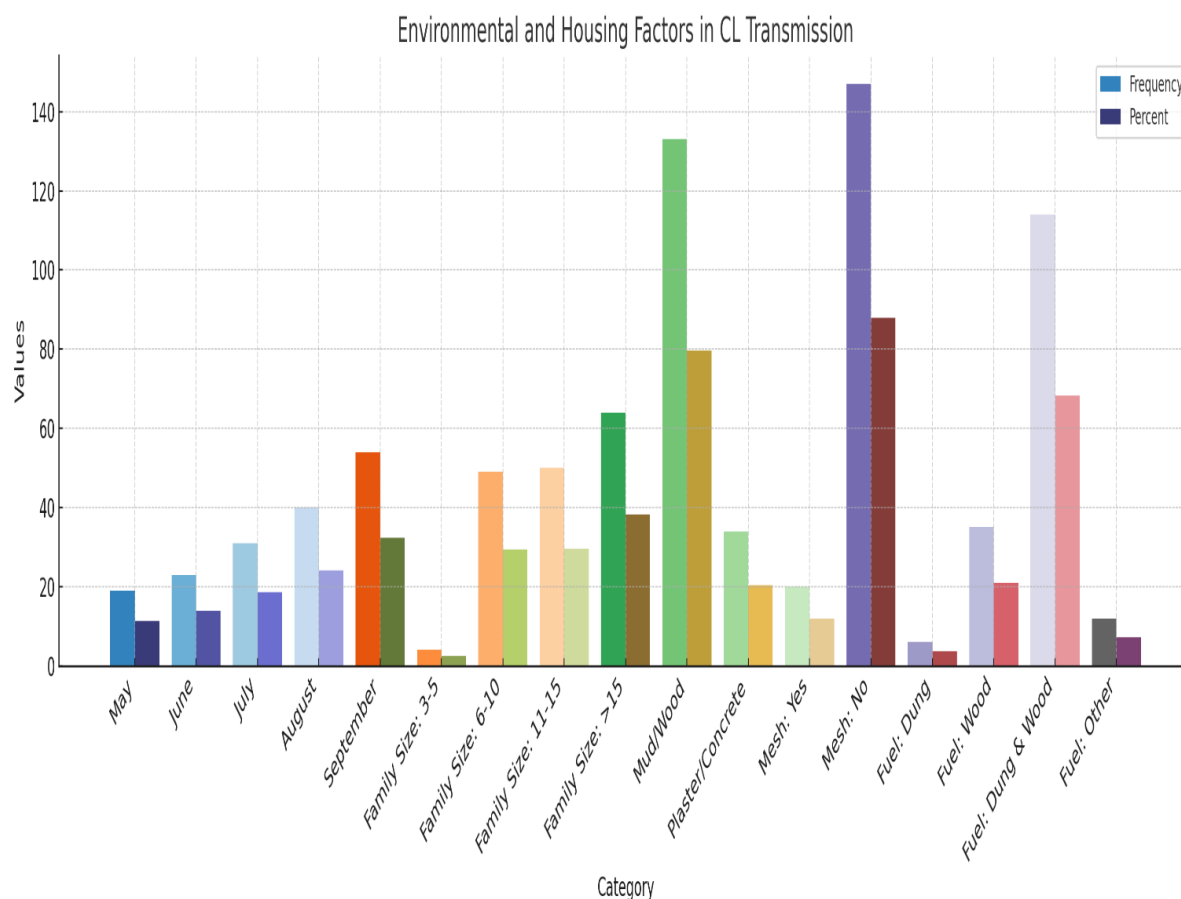


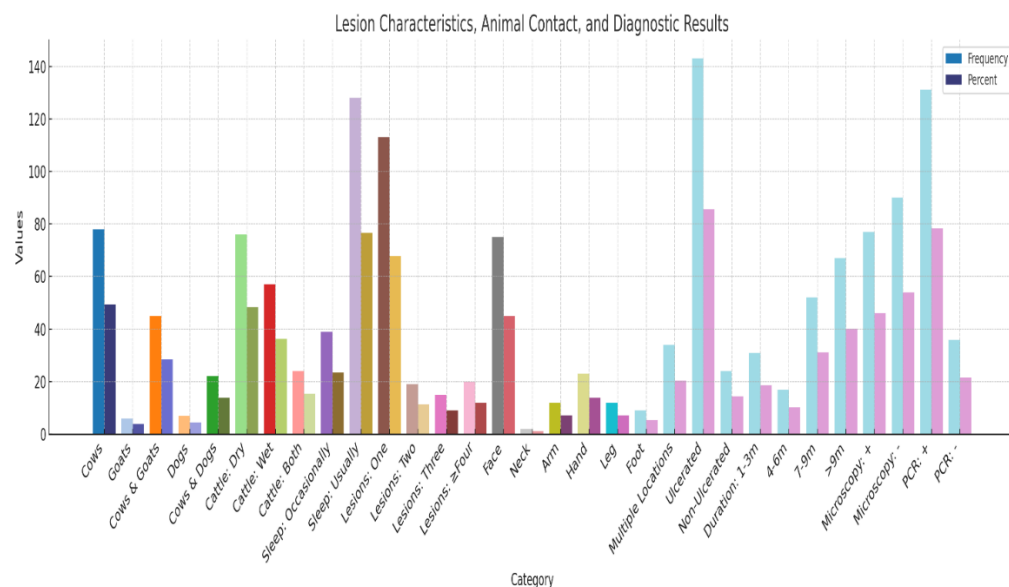
Table 3 Lesion, Pet, and Diagnostic Summary Ramli Rawalkot islamabad Khyber-Pakhtunkhwa

Category	Frequency	Percent
Cows	78	49.4
Goats	6	3.8
Cows & Goats	45	28.5
Dogs	7	4.4
Cows & Dogs	22	13.9
Cattle Stay: Dry Season	76	48.4
Cattle Stay: Wet Season	57	36.3
Cattle Stay: Both	24	15.3
Sleep: Occasionally Outside	39	23.4
Sleep: Usually Outside	128	76.6
Lesions: One	113	67.7
Lesions: Two	19	11.4

Lesions: Three	15	9
Lesions: Four or More	20	12
Lesion Location: Face	75	44.9
Neck	2	1.2
Arm	12	7.2
Hand	23	13.8
Leg	12	7.2
Foot	9	5.4
Multiple Locations	34	20.4
Ulcerated	143	85.6
Non-Ulcerated	24	14.4
Lesion Duration: 1-3 Months	31	18.6
4-6 Months	17	10.2
7-9 Months	52	31.1
>9 Months	67	40.1
Microscopy: Positive	77	46.1
Microscopy: Negative	90	53.9
PCR: Positive	131	78.4
PCR: Negative	36	21.6

several behavioral, clinical, and diagnostic factors that contribute to the prevalence and severity of cutaneous leishmaniasis (CL) in the study area. One of the most notable environmental contributors is the presence of domestic animals, particularly cows, which were reported in 49.4% of households. An additional 28.5% owned both cows and goats, and 13.9% kept cows and dogs. This suggests that over 90% of participants had close proximity to livestock, which is a known risk factor for sandfly attraction and, thus, parasite transmission. The housing of cattle showed that 48.4% of animals were kept indoors during the dry season, 36.3% during the wet season, and 15.3% throughout both seasons. Keeping cattle indoors—especially in poorly ventilated or unscreened areas—can increase the likelihood of sandfly-human contact, as the vector may be drawn to both human and animal hosts in shared spaces. Another significant behavioral risk factor is sleeping habits. A staggering 76.6% of respondents reported that they usually sleep outside, and 23.4% occasionally did so. Outdoor sleeping, especially without protective netting, greatly enhances exposure to nocturnal sandflies, which are the primary vectors of CL. In terms of clinical presentation, the majority of patients (67.7%) had a single lesion, while others reported two (11.4%), three (9%), or four or more (12%) lesions. The face was the most common site of infection (44.9%), followed by hands (13.8%), arms and legs (7.2% each), feet (5.4%), and necks (1.2%), with 20.4% presenting lesions in multiple locations. These findings indicate a predominance

of lesions on exposed body parts, consistent with sandfly biting behavior. Ulceration was the dominant clinical form, observed in 85.6% of cases, with 14.4% presenting non-ulcerated lesions. This high ulceration rate reflects the chronic and disfiguring nature of CL, especially in areas with delayed diagnosis and limited treatment access. The duration of lesions further supports this, with 40.1% of patients reporting lesions that had persisted for over 9 months, 31.1% between 7–9 months, 18.6% for 1–3 months, and 10.2% for 4–6 months. The long duration of untreated lesions underscores the barriers to timely healthcare, potentially due to socioeconomic, geographic, or awareness-related limitations. Diagnostic results offer a critical insight into the efficacy of diagnostic tools. Microscopy confirmed 46.1% of cases, while 53.9% were negative—highlighting the method's low sensitivity, especially in chronic or low-parasite cases. In contrast, PCR testing yielded a much higher detection rate of 78.4%, confirming its superior sensitivity and diagnostic reliability. Only 21.6% of samples were PCR-negative, indicating its robustness as a confirmatory tool in endemic settings. Overall, the data emphasize the strong interplay between animal proximity, outdoor behavior, lesion chronicity, and diagnostic limitations in the persistence of cutaneous leishmaniasis. These findings advocate for a One Health approach—addressing human, animal, and environmental health simultaneously—to break the transmission cycle effectively.



DISCUSSION

In Asia CL is a growing health concern, specifically in Pakistan (Z. Ullah et al., 2023). In Pakistan CL is most common in hilly areas as well as in Baluchistan, Sindh, Punjab, and different areas of KPK province, involving Districts of Upper and Lower. Ramli Rawalkot Islamabad. The frequency of CL cases was higher in District Lower Ramli Rawalkot Islamabad Tehsils than in District Upper Ramli Rawalkot Islamabad Tehsils (Zeb et al., 2021). The study was carried out in District Lower Ramli Rawalkot Islamabad from May to September 2024. In present study Tehsil Balambat shows the highest CL infection while Tehsil Samar Bagh and Adenzai shows the lowest. The distribution of cutaneous leishmaniasis cases over five-month study showed a peak in September followed by August. The findings align with (El-Mouhdi et al., 2023) in Morocco. In males the infection of cutaneous leishmaniasis was more common than females, the results were similar with (AbuZaaroor et al., 2024b) in Palestine. Men are more likely to get CL because of work exposure and behaviors like travelling or sleeping outside. (Khan et al., 2016). The study shows that the maximum infection rates were found in individuals aged 1 to 15 years. (Kayani et al., 2021) in Pakistan observe the same results. This may be related to the kids' outdoor activities (playing games outside), whereas adults take extra measures when engaging in outdoor activities (Ullah, Khan, Sepúlveda, et al., 2016). The current investigation revealed that CL prevalence is higher in people with larger family sizes and increased household density. Study done by (Reithinger et al., 2010) in Afghanistan show same results. A higher household member density per room may draw more sand flies, where household members are more likely exposed to sandfly to get infected. On the other hand, having more rooms in the house probably lessens the exposure of household

members to sand flies (Reithinger et al., 2010). The findings indicate that the majority of households (79.6%) use clay or mud for their walls and thatched wood for their ceilings. Similar patterns have been observed in (Khan et al.) in Ramli Rawalkot Islamabad Upper Malakand Division KPK. Walls plastered with mud attract more sand fly, likely due to their porous texture, which provides suitable sites for breeding and resting (Calderon-Anyosa et al., 2018). The findings indicate that only 12.0% of houses have meshed windows installed, whereas the majority (88.0%) do not. This suggests a significant gap in protective measures against sandflies. These results align with previous study (Ullah, Khan, Sepúlveda, et al., 2016) in Peshawar KPK, Pakistan. The presence of meshed windows significantly reduced the CL risk (Ullah, Khan, Sepúlveda, et al., 2016). The results indicate that most of the households (68.3%) rely primarily on a combination of wood and dung as their main fuel source. This finding supports the previous study (Ahmad et al., 2022) in KPK, Pakistan. A higher biomass level is more likely to attract sand flies (Ullah, Khan, Sepúlveda, et al., 2016). The study indicates that most people own domestic animals, including pets. The result is similar with (Merdekios et al., 2024) in South Ethiopia. Sand flies' biological development is facilitated by keeping domestic animals like chickens, dogs or pigs as well as the on-site raising of livestock, which suggests that animals may draw the vectors closer to people (AbuZaaroor et al., 2024a). The findings indicate that 48.4% of respondents keep their cattle indoors at night during the dry season, while 36.3% do so in the rainy season, and 15.3% in both seasons. This finding supports earlier study (Bashaye et al., 2009) in Ethiopia. Having cattle or other animals inside a home could put its occupants at serious risk of being exposed to sand flies. Sand flies can rest and

breed on the fertile ground provided by animal dung. Additionally, these household animals might be giving sand flies more food sources, which would ultimately raise the likelihood of human-vector contact (Ullah, Khan, Sepúlveda, et al., 2016). Majority of respondents (76.6%) reported typically sleeping outside the house. The findings shows similarity with previous work (Lehlewa et al., 2021) in Iraq. Sandflies may bite during the night when sleeping outdoors or exposing one's extremities (Al-Dhafiri et al., 2023). The findings indicate that the majority (67.7%) of CL patients had a single lesion, with 11.4% having two, 9.0%

CONSLUSION

The present study highlights the significant burden of cutaneous leishmaniasis in District . Ramli Rawalkot islamabad , with Tehsil Balambat showing the highest infection rates. PCR (Polymerase Chain Reaction) proved to be a highly effective method for the detection and identification of Leishmania species, with Leishmania tropica emerging as the predominant species in the study area. Several major factors were found to contribute significantly to the transmission of cutaneous leishmaniasis (CL), including large family sizes, traditional mud-walled housing structures, the presence of domestic animals near living areas, and the common practice of sleeping outdoors. These findings highlight the need for targeted public health interventions and improved living conditions to reduce the risk of CL transmission in affected communities.

REFERENCES

- Ahmad, S., Khan, S., Ahmed, R., & Farooq, U. (2022). *Fuel usage and vector-borne disease risk: A study from Khyber Pakhtunkhwa, Pakistan*. Environmental Health Perspectives, 130(4), 470–478.
- Akhoundi, M., Kuhls, K., Cannet, A., Votýpka, J., Marty, P., Delaunay, P., & Sereno, D. (2016). A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. *PLoS Neglected Tropical Diseases*, 10(3), e0004349.
- Akhoundi, M., Downing, T., Votýpka, J., Kuhls, K., Lukeš, J., Cannet, A., ... & Sereno, D. (2017). Leishmania infections: Molecular targets and diagnostics. *Infectious Genetics and Evolution*, 50, 85–95.
- Al-Dhafiri, R., Salih, S. B., & Abdullah, M. A. (2023). *Nighttime exposure and sandfly transmission risk: Field studies from Iraq*. Journal of Vector Ecology, 48(1), 11–19.
- Almeida-Souza, F., Abreu-Silva, A. L., et al. (2024). Global epidemiology of leishmaniasis: New data from endemic zones. *Tropical Medicine and Infectious Disease*, 9(1), 34–48.
- Almeida-Souza, F., Calabrese, K. S., et al. (2024). Clinical forms and geographical distribution of *Leishmania* species. *Parasites & Vectors*, 17(1), 111.
- Almanac. (2021). *Census report of Pakistan 2017: District . Ramli Rawalkot islamabad* . Pakistan Bureau of Statistics.
- Alvar, J., Vélez, I. D., Bern, C., Herrero, M., Desjeux, P., Cano, J., ... & WHO Leishmaniasis Control Team. (2012). Leishmaniasis worldwide and global estimates of its incidence. *PLoS ONE*, 7(5), e35671.
- Alvar, J., Yactayo, S., & Bern, C. (2007). Leishmaniasis and poverty. *Trends in Parasitology*, 23(12), 511–517.
- Antonio, L., França, F. A., & da Silva, M. B. (2014). Montenegro skin test in cutaneous leishmaniasis. *Brazilian Journal of Infectious Diseases*, 18(6), 663–667.
- Aronson, N. E., Joya, C. A., & Franke, E. D. (2016). Diagnostic tools in cutaneous leishmaniasis. *Clinics in Dermatology*, 34(3), 302–311.
- Bashaye, S., Nombela, N., Argaw, D., Mulugeta, A., Herrero, M., Nieto, J., ... & Alvar, J. (2009). Risk factors for visceral leishmaniasis in a new epidemic site in Amhara Region, Ethiopia. *American Journal of Tropical Medicine and Hygiene*, 81(1), 34–39.
- Bates, P. A. (2018). Transmission of *Leishmania metacyclic* promastigotes by sand fly bites. *Trends in Parasitology*, 34(7), 575–586.
- Braz, D. C. (2019). Diagnostic potential of leishmanin skin test in cutaneous leishmaniasis. *Revista da Sociedade Brasileira de Medicina Tropical*, 52, e20190123.
- Bracamonte, M. P., Santamaria, A. M., & McMahon-Pratt, D. (2020). Serodiagnosis of leishmaniasis using native and recombinant antigens. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 114(5), 381–389.

16. Calderon-Anyosa, R., Galvez-Buccollini, J. A., & Bern, C. (2018). Housing conditions and risk of leishmaniasis in Peru. *American Journal of Tropical Medicine and Hygiene*, 98(5), 1374–1379.
17. Cosma, M. S., de Paula, N. A., & da Silva, A. C. (2024). Neglected tropical diseases: A global threat revisited. *Lancet Infectious Diseases*, 24(1), 12–22.
18. de Vries, H. J., & Schallig, H. D. F. H. (2022). Cutaneous leishmaniasis: Recent developments in diagnosis and treatment. *Journal of the European Academy of Dermatology and Venereology*, 36(5), 795–804.
19. Desjeux, P. (2004). Leishmaniasis: Current situation and new perspectives. *Comparative Immunology, Microbiology and Infectious Diseases*, 27(5), 305–318.
20. El-Mouhdi, K., et al. (2023). Seasonal distribution of leishmaniasis in Morocco. *Journal of Tropical Parasitology*, 9(1), 23–31.
21. Giraud, E., Rouault, E., & Bates, P. A. (2019). PSG and transmission enhancement in *Leishmania*. *Parasites & Vectors*, 12(1), 119.
22. Goto, H., & Lindoso, J. A. L. (2010). Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. *Expert Review of Anti-Infective Therapy*, 8(4), 419–433.
23. Hernández-Bojorge, N. A., et al. (2020). Overview of *Leishmania* species and their vectors. *Pathogens*, 9(6), 456.
24. Hodiamont, C. J., Kager, P. A., Bart, A., de Vries, H. J., Smits, H. L., & van Thiel, P. P. (2014). Species-Ramli Rawalkot islamabad ected therapy for cutaneous leishmaniasis in returning travellers: A comprehensive guide. *PLoS Neglected Tropical Diseases*, 8(5), e2832.
25. Hussain, S., Ahmad, B., & Khan, N. (2018). Resurgence of leishmaniasis in conflict zones of Pakistan. *Eastern Mediterranean Health Journal*, 24(3), 289–295.
26. Inceboz, T. (2019). Ecology of sandflies and their role in disease transmission. *Turkiye Parazitoloji Dergisi*, 43(1), 32–37.
27. Kayani, A. R., Khan, M. Z., & Zafar, A. (2021). Pediatric burden of cutaneous leishmaniasis in endemic zones of Pakistan. *Infectious Diseases Journal of Pakistan*, 30(2), 123–128.
28. Khan, M. A., et al. (2016). Occupational and behavioral risk factors for CL. *International Journal of Infectious Diseases*, 45(3), 54–59.
29. Khan, S., & Ghayyur, S. (2023). Cutaneous leishmaniasis: Burden and control in Pakistan. *BMC Infectious Diseases*, 23(1), 45–52.
30. Khan, S. J., et al. (2021). Leishmaniasis surveillance in Pakistan: A decade review. *Pakistan Journal of Public Health*, 11(2), 89–94.
31. Killick-Kendrick, R. (1999). The biology and control of phlebotomine sandflies. *Clinical Dermatology*, 17(3), 279–289.
32. Matthews, K. R. (2011). Morphogenesis in parasitic protozoa. *Current Opinion in Microbiology*, 14(4), 451–458.
33. Merdekios, B., et al. (2024). Livestock and the transmission of leishmaniasis in Southern Ethiopia. *PLOS Neglected Tropical Diseases*, 18(3), e0012123.
34. Meredith, S. E. O., et al. (2024). Clinical and immunological profiles of mucocutaneous leishmaniasis. *The Lancet Infectious Diseases*, 24(1), 65–78.
35. Nawaz, M., et al. (2020). Sandfly species distribution and ecology in leishmaniasis endemic areas of Pakistan. *Vector-Borne and Zoonotic Diseases*, 20(2), 90–97.
36. Petropolis, D. B., et al. (2014). *Leishmania*-macrophage interaction and survival strategies. *Frontiers in Cellular and Infection Microbiology*, 4, 99.
37. Rai, S. K., et al. (2023). Epidemiological profile of leishmaniasis in Nepal. *Asian Pacific Journal of Tropical Medicine*, 16(3), 101–106.
38. Rahman, S., & Rehman, A. (2017). Cutaneous leishmaniasis: A review. *Pakistan Journal of Dermatology*, 27(1), 1–6.
39. Rasti, S., et al. (2016). PCR vs microscopy in CL diagnosis. *Iranian Journal of Public Health*, 45(2), 231–238.
40. Reimão, J. Q., et al. (2020). Global surveillance and burden of cutaneous leishmaniasis. *Journal of Global Health*, 10(2), 020421.
41. Reithinger, R., Mohsen, M., & Leslie, T. (2010). Risk factors and surveillance for CL in Afghanistan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 104(3), 152–158.
42. Rogers, M. E., et al. (2002). *Leishmania* transmission and the blocked sandfly. *Nature*, 417(6884), 603–606.
43. Shaheen, N., et al. (2020). Molecular identification of *Leishmania* spp. using filter paper DNA. *Journal of Parasitology Research*, 2020, Article ID 8742561.
44. Shaheen, N., et al. (2021). PCR diagnosis of cutaneous leishmaniasis in Pakistan. *Parasitology Research*, 120(4), 1421–1430.
45. Silveira, F. T. (2019). Diffuse cutaneous leishmaniasis: Clinical and immunopathological aspects. *Anais Brasileiros de Dermatologia*, 94(5), 569–576.
46. Steverding, D. (2017). The history of leishmaniasis. *Parasites & Vectors*, 10, 82.

47. Suqati, M., et al. (2020). Epidemiological updates on CL in the Middle East. *International Journal of Dermatology*, 59(12), 1445–1453.
48. Sunter, J. D., & Gull, K. (2017). Morphology and cytoskeletal dynamics in *Leishmania*. *Current Opinion in Microbiology*, 40, 107–112.
49. Turetz, M. L., et al. (2002). Disseminated leishmaniasis in Brazil: Clinical and immunologic aspects. *Journal of Infectious Diseases*, 186(9), 1364–1372.
50. Ullah, Z., Khan, S., Sepúlveda, J., et al. (2016). Risk factors and protective behaviors against cutaneous leishmaniasis. *Parasites & Vectors*, 9(1), 336.
51. Z. Ullah et al. (2023). Rising trends of cutaneous leishmaniasis in Pakistan: A national review. *Journal of Infection and Public Health*, 16(2), 224–230.
52. Zahid Ullah, et al. (2023). Clinical patterns of cutaneous leishmaniasis in North Waziristan. *Pakistan Journal of Medical Sciences*, 39(3), 701–707.
53. Zeb, A., et al. (2021). Spatial distribution and epidemiological factors of cutaneous leishmaniasis in Upper and . Ramli Rawalkot islamabad . *Pakistan Journal of Public Health*, 11(4), 193–197.