# An update on clinical and pathogenic spectra of leishmaniasis

Angamuthu Selvapandiyan[1*], Shital Shital[1], Diya A'gitok Sangma[2], Manju Jain[2], Nadira Karunaweera[3] and Nirmal K. Ganguly[4*]

[1]Department of Molecular Medicine, Jamia Hamdard, New Delhi 110062, India
[2]Department of Biochemistry, Central University of Punjab, Bathinda, Punjab, 151401, India
[3]Department of Parasitology, Faculty of Medicine, University of Colombo, Colombo-08, Sri Lanka
[4]Department of Research, Sir Gangaram Hospital, New Delhi, 110060, India
*Corresponding authors: Angamuthu Selvapandiyan: email: selvapandiyan@jamiahamdard.ac.in; Nirmal K. Ganguly: ganguly1nk@gmail.com

## Abstract

Leishmaniasis, classified as a neglected tropical disease, exerts its impact on millions globally. Its clinical spectrum encompasses diverse forms, from benign self-resolving skin lesions (Cutaneous leishmaniasis) to life-threatening visceral infections (Visceral leishmaniasis or kala-azar). This review aims to comprehensively explore the spectrum of the disease as an outcome of often-overlooked parasite variants. Additionally, it addresses the emerging challenges faced in the pursuit towards disease elimination. The evolving landscape of leishmaniasis demands development of molecular surveillance tools for detection of the heterogeneous parasite strains that contribute to the emergence of new endemic foci. Such surveillance poses formidable challenges to current elimination strategies. As the disease landscape continues to evolve, understanding the molecular intricacies of causative parasite strains becomes paramount. This knowledge not only aids the understanding of the basis of emerging/shifting endemic areas but also facilitate the search for and the design of targeted interventions. In this context, the review will navigate through the dynamic terrain of leishmaniasis, the various causative species of *Leishmania* parasites emphasizing the urgency



DOI: 10.1017/erm.2025.4

for development of robust surveillance mechanisms and innovative approaches to confront the evolving challenges in our quest for global disease elimination.

**Keywords:** Clinical challenges; Visceral leishmaniasis; Cutaneous leishmaniasis; Mucocutaneous leishmaniasis; Coinfections; Molecular tools

## Introduction

Leishmaniases is a group of diseases that occur in humans and in other mammals in the tropical and subtropical regions of world (in 90 countries: www.cdc.gov/parasites/leishmaniasis), with a prevalence in countries around the Mediterranean basin, parts of Africa, Asia, and Central and South America [1]. It is caused by protozoan parasitic species of the genus *Leishmania* and spread by the bite of the insect sand fly, *Phlebotomus* spp. The disease manifests in several clinical forms, ranging from self-healing skin lesions (cutaneous leishmaniasis; CL) to potentially fatal visceral infections (visceral leishmaniasis [VL] or kala-azar). CL typically results in ulcers on the skin, while VL affects internal organs such as the liver, spleen, and bone marrow. The disease affects millions of people worldwide, particularly those living in poverty, with compromised immune systems, or in areas with inadequate healthcare infrastructure. This review article delves into the various forms of leishmaniases, including those less commonly discussed, alongside the emerging challenges we encounter in efforts towards its elimination.

## Taxonomy of *Leishmania*

Genus *Leishmania* belongs to the family Trypanosomatidae in the order Kinetoplastida within the class of Euglenozoa, a phylum of Protista. The family Trypanosomatidae encompasses a diverse group of flagellated protozoan parasites, including the genera *Trypanosoma* and *Leishmania*, which cause diseases such as trypanosomiasis and leishmaniasis, impacting human and animal populations globally [2]. The order Kinetoplastida is a group of single-celled parasitic protozoa characterized by the presence of a distinctive DNA-containing structure called a kinetoplast. We present here the disease dynamics with the various species/strains of *Leishmania* causing several clinical manifestations in humans/mammals and the regions globally affected by the parasite variants depicted in **Figure 1 and Table 1**.



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

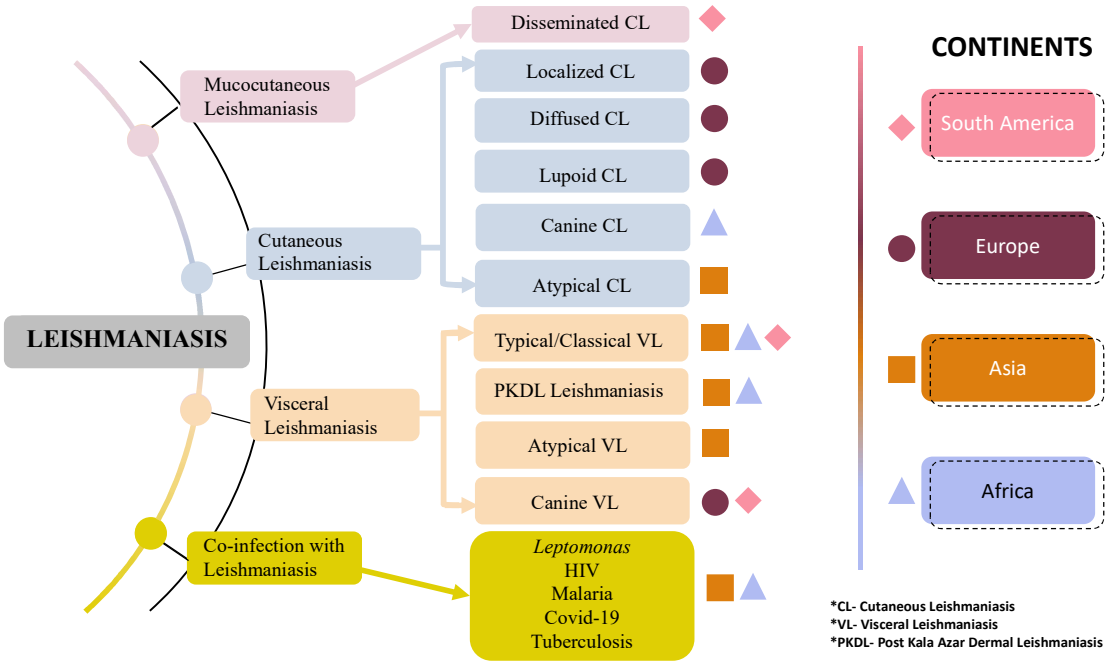



**Figure 1:** The sketch describes various forms of leishmaniases and the continents where each of the diseases is prevalent.

**Table 1:** Table describes various forms of leishmaniases and their causative *Leishmania* species along with the regions globally affected, symptoms and challenges towards /treatment elimination strategies.

| S. No. | Type of leishmaniasis | *Leishmania* sp. | Region affected | Clinical symptoms | Challenges in eradication |
|---|---|---|---|---|---|
| 1 | Visceral | *L. donovani, L. infantum, L. chagasi* | Bangladesh, Brazil, India, Ethiopia, Sudan, Nepal, and South Sudan [3]. | Fever, Weight loss, and Organomegaly, Lymphadenopathy, Anemia, thrombocytopenia, and neutropenia, Hyperglobulinemia, Hepatic dysfunction, jaundice, and ascites [4,5]. | Poor vector control strategies, limited diagnostic services, drugs, treatments and lack of community awareness [6]. |
| 2 | Cutaneous | *L. major, L. tropica, L. aethiopica, L. braziliensis, L. mexicana, and L. panamensis.* | Afghanistan, north and West Africa, Brazil, Colombia, South America, Iran, Middle East, Central Asia [3]. | Ulcerating lesions, single or multiple, palpable lymph nodes [3]. | Treatment failure due to various factors like age, number and size of lesions [7]. |



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

| | | | | | |
|---|---|---|---|---|---|
| 3 | Muco-cutaneous | *L. tropica, L. major, L. donovani, L. infantum L. vianna (V) subgenus, L. (V) brazilensis, L. (V) panamensis, L. (V) guyanesis, L. (V) peruviana, L. amazonensis* [8] | Germany, Central and South America [9,10] | Nasal secretions, nasal obstruction, pain, epistaxis. Destructive lesions in nose, oropharynx. Initially involves nose and mouth, can progress to include pharynx and larynx [8]. | Diagnosis of oral leishmaniasis is a challenge mainly because it is rarely encountered [11], primarily due to lack of laboratory setting. |
| 4 | Post-Kala-Azar Dermal | *L. donovani, L. infantum, L. tropica* [12,13]. | India, Sudan, South Sudan, Bangladesh, Ethiopia, Nepal, Mediterranean countries and Latin America [12,13]. | Hypopigmented patches, erythematous succulent papulo-plaques, and nodular lesions on the face and upper body, genitalia, and tongue. Atypical symptoms-photosensitivity, verrucous, hypertrophic, xanthomatous, and ulcerative lesions [14]. | Unseeking of treatment as symptoms are minimal. Thus, act as reservoir [15,16]. |
| 5 | Diffuse cutaneous | *L. amazonensis* [17] | Brazil [17] | Diffuse papulonodular eruption on trunk and upper extremities [18]. | Resistant to chemotherapy and also is associated with absent cell-mediated response [19]. |
| 6 | Lupoid | *L. tropica* | Pakistan [20], Afghanistan, Africa and Europe [21]. | Erythematous and infiltrated plaque , psoriasiform [22], ulcerated/crusted and Discoid lupus erythematosus like, solitary plaque extending over the face and multiple lesion [20]. | Microscopic examination of direct smears has low sensitivity to diagnose. $CO_2$ laser radiation based treatment seems efficient for LCL [23]. |
| 7 | Localized cutaneous | *L. mexicana*, *L. tropica* [24]. | Afghanistan, Nicaragua, Algeria, Brazil, Syrian Arab Republic, Honduras, Iran, Pakistan, Peru, Colombia, Saudi Arabia, Tunisia, Turkey, | Single papular or nodular skin lesion that progressively ulcerates [26]. | Severe pain due to papular or nodular skin lisions due to delay in diagnosis and treatment is a major challenge. |



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

| | | | | | |
|---|---|---|---|---|---|
| | | | Morocco and Yemen [25]. | | |
| 8 | Disseminated | *L. panamensis* *L. guyanensis* | Subtropical and tropical lowlands of the Pacific coastal region [27,28]. | Disseminated pleomorphic ulcers, papules, and cutaneous plaque-like lesions [29]. | DCL has been poorly studied. The failure rate to pentavalent antimony therapy is 75% [30]. |
| 9 | Atypical | *CL-* *L. donovani com plex*, *L. infantum, L. chagasi* VL- *L. tropica complex* *L. amazonensis, and L. major* [31] | Mediterranean Region, North Africa, Europe, America [32]<br><br>Bahia, Brazil, Saudi Arabia, Iran, Kenya, Israel and India [33]. | Same as typical VL.<br><br>Same as typical CL. | Cases of atypical forms of the disease in all types of leishmaniases pose a considerable challenge for clinicians during the initial screening of the disease [34]. |
| 10 | Canine | *L. (V) braziliensis, L. (V) chagasi, L (V.) panamensis, L (V.) peruviana L (V.) guyanensis* [35] *L. infantum* [36] | Marmara, Ege, Black Sea and Mediterranean regions of western Turkey [36] | Local, ulcerative lesions on the nipples, scrotum, ears, feet and muzzle [35]. | Canine leishmaniasis serve as reservoir hosts of VL [1,37]. |

## Cutaneous Leishmaniasis (Typical)

CL, the most prevalent form of the disease that is characterized by skin lesions and ulcers caused by *Leishmania* species, particularly *Leishmania braziliensis, L. guyanensis, L. panamensis, L. peruviana, L. mexicana*, and *L. amazonensis* in the New World and the *L. tropica, L. major*, and *L. infantum* complexes in the Old World [38]. CL is traditionally known as 'oriental sores' and typically occurs at the site of inoculation. Transmitted through the bite of infected phlebotomine sand flies, this parasitic infection often results in self-healing lesions within six months without intervention. The specific ulcerating granuloma of skin is an initial papule, later transforming into ulcer [39]. The non-healing skin ulcers, especially on the face or limbs, can lead to considerable morbidity. The global burden is substantial, with an estimated 700,000 to 1 million new cases reported annually worldwide [40]. The incubation period between an infected sand fly bite and lesion development ranges from 2 weeks to 6 months. Despite the self-limiting nature of most cases, the persistence of non-healing ulcers underscores



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

the potential for significant public health impacts, emphasizing the importance of ongoing research and interventions to address this widespread and debilitating disease.

Apart from the parasite species/variant involved, the disease prognosis and outcome in terms of number of lesions, types of lesions, extent of host tissue damage and lesional parasite load depends on the host immune response to parasite specific antigens [41]. On this line, there is a risk of dissemination in immunodeficient patients with prolonged illness and the disease may take a chronic form. Different modalities of cutaneous disease outcome are discussed in following sections.

## Localised cutaneous leishmaniasis

Localised cutaneous leishmaniasis (LCL) may be caused by several species of *Leishmania* with the lesion(s) occurring at the site of the insect bite. The incubation period for this form ranges from 1 to 4 weeks and can last for up to several years [42]. The affected body sites are the ears, nose, upper lip, cheeks, legs, hands, and forearms.

Typical clinical manifestation for this form of disease is appearance of an erythematous but painless papule that is non-itchy. It may transform into a nodule or an ulcer with nodular or thick borders having sharp and elevated edges. LCL can heal spontaneously in 3–9 months in the case of *L. mexicana*, 2–6 months in the case of *L. major*, and 6–15 months for *L. braziliensis*, *L. tropica*, or *L. panamensis* infection. There are high chances of relapse, with similar or more severe clinical manifestations than those observed at the initial episode [41].

## Diffuse cutaneous leishmaniasis

In rare incidences of CL, diffuse cutaneous leishmaniasis (DCL) may arise, characterized by non-ulcerating nodules affecting large skin areas, causing prolonged and severe disabilities persisting for months or even years [43]. This is characterized by an anergic of cellular immune response to parasite antigens [44]. The disease is disseminated through tissue, lymph, and blood giving rise to widespread skin lesions. It often starts with hard erythematous nodules and reddish-brown infiltrative smooth or verrucous plaques. The disease phenotype is observed in



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

Amazonian Brazil, Central America, Ethiopia, Kenya and Venezuela, and is caused by the *L. mexicana* complex (*L. amazonensis*, *L. braziliensis* and *L. pifanoi*). With a poor T cell response, lesions exhibit a larger number of parasitized macrophages in DCL [45]. This clinical form is generally resistant to treatment. There is no spontaneous resolution, and a prolonged disease of up to 20 years has been observed.

**Disseminated cutaneous leishmaniasis**

The occurrence of multiple polymorphic cutaneous lesions distributed over more than two non-contiguous parts of the body is described as disseminated cutaneous leishmaniasis (DSL). It occurs less frequently and is mainly seen in the new world region [30] In almost half of the cases, an association with nasal mucosal lesions had been observed. In the phylogenetic analysis of Cytochrome b gene sequences of various species of *Leishmania*, the *Leishmania* strain that causes DSL was observed among the group responsible for MCL and more closely placed with *L. guyanensis* [45], which is also commonly observed in the New World. Classically CL, DSL and MCL are grouped as American tegumentary leishmaniasis (ATL) in the Americas, especially due to *L. braziliensis* [46].

**Lupoid leishmaniasis**

Lupoid leishmaniasis (LS) is also referred as leishmaniasis Rucidivans (LR), and is a rare, cutaneous form of leishmaniasis, occurring in patients with a good cellular immune response [21]. It is caused due to recurrence of cutaneous disease at the sites of previously cured CL lesions. LR detection is hard as it is different from acute lesions, owing to the absence of parasites in tissue biopsies of the lesion [47]. It is mostly caused by *L. tropica* in the Old Word.

**Mucocutaneous Leishmaniasis**

Mucocutaneous leishmaniasis (MCL) is a less common form of leishmaniasis that can result in partial or complete destruction of the mucous membranes in the nose, mouth, and throat [48]. Clinically, there is an early infiltration of the mucosa with superficial ulcerations, borders



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

having a necrotic appearance that are torn and detached. The uvula, pillars of the palate roof, and tonsils are often destroyed. This condition can occur as a consequence of infection with certain species of the leishmaniasis parasite that cause CL in parts of Latin America. Some types of the parasite can spread from the skin and cause sores in the mucous membranes of the nose (most commonly), mouth, or throat. MCL is a destructive form of leishmaniasis, only seen with the American species of *Leishmania* (Viannia subspecies), which includes *L. braziliensis*, *L. guyanensis*, and *L. panamensis*. The enhanced co-lateral tissue damage involved is due to the elevated inflammatory immune-response with a low immune-regulatory mechanism in place [41]. According to the CDC, this condition mainly affects individuals in Bolivia, Brazil, Ethiopia, and Peru, with over 90% of cases occurring in these countries [24]. The diagnosis of oral leishmaniasis is challenging primarily due to its rare occurrence in settings lacking sufficient laboratory support and appropriate testing capabilities.

**Visceral Leishmaniasis (Typical)**

The systemic Visceral leishmaniasis (VL) disease form, commonly known as kala-azar (KA) or dum-dum fever, is a life-threatening disease listed among neglected tropical diseases by the World Health Organization (WHO) [49]. If left untreated, VL proves fatal in over 95% of cases. This debilitating condition primarily targets the internal visceral organs, such as the liver, spleen, and bone marrow. VL is known to induce hyperplasia of reticulo-endothelial cells of organs involved. Clinical manifestation of the disease includes anorexia, lymphadenopathy, hepatomegaly, splenomegaly, pallor, anemia, thrombocytopenia, fever, weakness, cutaneous pigmentation, and weight loss, which progresses rapidly in weeks or months. The incubation period of the disease is from 3 to 8 months. Children have prominent symptoms than adults in many areas, and the disease progresses rapidly in people with a weakened immune system, particularly those with AIDS, than in people with a healthy immune system.

*L. donovani* is the causative agent in the Indian subcontinent, Asia, and Africa, affecting both adults and children. In the Mediterranean region, southwest and central Asia, and South America, particularly in young children, VL is caused by *L. infantum* or *L. chagasi*. While significant progress has been made in many regions, the disease persists as a major health concern in east Africa, Southeast Asia, and Brazil [50]. India accounts for 18% of the global burden of VL in 2020. It is present in 54 districts across four endemic states in India: Bihar (33 out of 38 districts), Jharkhand (4 out of 24 districts), Uttar Pradesh (6 out of 75 districts) and



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

West Bengal (11 out of 23 districts) [51]. Sporadic cases are also reported in other states including Assam, Gujarat, Himachal Pradesh, Jammu & Kashmir, Kerala, Madhya Pradesh, Haryana, Puducherry, Sikkim, Tamil Nadu and Uttaranchal [51].

WHO's global leishmaniasis surveillance for 2017–2018, along with additional indicators, underscores the continued importance of monitoring and addressing VL. An estimated 50,000 to 90,000 new cases emerge annually worldwide [40]. This stark prevalence emphasizes the urgency of sustained efforts to control the disease, particularly in regions where it continues to impact vulnerable populations. The global health community's commitment to tackling VL remains crucial in preventing its high mortality rate and reducing the burden on affected communities [1].

KA elimination approach and strategies have witnessed a huge upsurge. Due to intense control and elimination strategies in the country, KA cases have decreased by 98% (1,275 cases in 2021) since the start of intensified activities in 1992 (77,102 cases). To get to the 2030 Sustainable Development Goals and WHO targets for kala-azar elimination, the block level incidence of cases needs to be reduced to less than 1 case per 10,000 population. This target aligns with the new NTDs roadmap 2021-2030 [1,52]. By the end of 2021, 98% of blocks have achieved the WHO elimination threshold.

India is redoubling its efforts to resolve known and newer challenges of under-reporting, detection of asymptomatic cases, Post-Kala-Azar Dermal Leishmaniasis (PKDL: described separately below), atypical leishmaniasis cases and emergence of newer endemic zones in the elimination of VL [53]. India has hugely expanded vector control interventions. The endemic states need to mandatorily notify cases to National Vector Borne Disease Control Programme (NVBDCP) every month, even if there are zero cases [54]. In recent years of KA, India has witnessed about 97% reduction of VL cases largely due to the introduction of single-dose AmBisome. In endemic villages that have reported cases of kala-azar over the past 3 years, 2 rounds of indoor residual spraying are being applied. WHO in coordination with the Ministry of Health and Family Welfare, Government of India and NCVBDC organized a coordinated program to assess the situation and progress of the KA elimination program in two endemic states, West Bengal and Uttar Pradesh [55]. PKDL is a sequel of VL in certain populations following apparent cure of VL [14]. PKDL patients harbour the parasite in skin lesions and may be the source of new infection to vectors even after two decades of eliminating the disease. Focused efforts on control of PKDL cases along with recent challenge of cutaneous cases



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

caused by *L. donovani* variants are being recognized as existing source of parasite in circulation that can lead to newer cases of VL upsurge.

**Post Kala-azar Dermal Leishmaniasis**

PKDL is a disease form of concern as a cutaneous sequel following VL or kala-azar. It is a form of CL that usually occurs months to years after treatment of VL [14]. It typically manifests six months to a year or more after kala-azar that is assumed to have been cured, however, it can happen even earlier. It typically manifests as hypopigmented macular, papular and nodular rash. People with PKDL are considered a potential source of *Leishmania* infection. While it typically emerges as a sequalae of VL, intriguingly, some individuals exhibit PKDL symptoms without a prior history of VL. First described by Dr. U. N. Brahmachari in 1922, the condition was termed "dermal leishmanoid" [56]. The symptoms of PKDL encompass a variable combination of hypopigmented patches, erythematous succulent papulo-plaques, and nodular lesions, primarily on the face and upper body, and occasionally extending to the extremities, genitalia, and tongue. Recent documentation indicates a notable decrease in the interval between VL and PKDL, with over 35% of cases presenting within just one year after a bout of VL, adding complexity to the understanding of the disease progression [14].

Leprosy and PKDL resemble closely in their clinical manifestations. A rapid accurate assay called 'm-LAMP' could be used for the differential diagnosis of leprosy versus PKDL [57]. In a comparison of treatment susceptibilities between VL and PKDL isolates, the latter displayed reduced susceptibility to miltefosine than the VL isolates [58]. Towards that end a combination therapy with liposomal amphotericin B and miltefosine displayed larger efficacy in healing of PKDL [59]. Correct diagnosis and timely treatment of PKDL is the next important milestone to be achieved in the consolidation phase of VL elimination operational in South-East Asia.

**Para-Kala-azar Dermal Leishmaniasis**

Para-kala-azar dermal leishmaniasis (Para-KDL) is an evolved condition associated with the presence of both PKDL and VL [60,61]. Despite cross-sectional studies revealing only 16 cases from 2012 to 2021, these cases were successfully treated and cured using high doses of Liposomal Amphotericin B (20 mg/Kg) in Bangladesh [61]. Active prevalence of para-KDL



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

has been reported in East Africa, although rare cases have also been documented in India and Brazil [62,63]. A study identified nine cases in India, mainly from Bihar, linked to relapse from miltefosine treatment [62]. The challenges of diagnosing Para-KDL contribute to poor prognoses for affected individuals, particularly as parasites show reduced susceptibility to current treatments [61]. To explore this issue, genome sequencing was conducted on sodium stibogluconate (SSG)-sensitive and resistant *L. donovani* strains, revealing 24 unique mutations in Para-KDL strains that may contribute to their dermatotropic behaviour [60]. Interestingly, 3 cases of HIV-para-KDL comorbidities were also observed in Brazil [64]. These findings highlight the need for ongoing monitoring and secondary prophylaxis in patients with VL.

**Atypical leishmaniasis**

The association between the infecting *Leishmania* species, more importantly the VL causing *L. donovani* and its clinical outcome appears to be modifying in the recent years with the emergence of newer parasite variants and disease occurrence in newer regions. Such atypical forms in Sri Lanka include cutaneous lesions that exhibit unusual characteristics, as well as cases presenting with systemic symptoms not typically associated with VL [65-69]. Similar cases had been reported from Himachal regions of India and other northern neighbouring countries [31,70-72]. The changing disease landscape warrants detailed molecular surveillance of the heterogeneous parasite populations that emerge in new endemic sites posing challenges to the disease elimination strategies. Kinetoplast DNA based phylogenetic analysis reveals distinct differences between VL causing *L. donovani* and CL causing *L. donovani* variants [73]. Whole genome sequence analysis also has shed considerable light on genetic variations and polymorphisms that exist between causative parasites in different regions [70]. Interestingly, *L. donovani* that causes CL in Sri Lanka has been placed a considerable distance from the CL causing other *Leishmania* species in phylogenetic analysis [74]. In Himachal Pradesh as the new endemic site for CL caused by *L. donovani*, the parasite isolates from CL patients comprise considerable heterogeneity at genetic level with accumulation of wide genetic mutations in terms of ploidy changes, copy number variations, InDels and SNPs that are different from those detected for *L. donovani* CL isolates from Sri Lanka [70].



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

On a similar note, the atypical phenotype caused by *L. donovani* is further exemplified through reports on MCL cases in Sri Lanka and India due to *L. donovani* [75,76]. There were also several studies in the past describing the viscerotropic (VL causing) nature of *L. tropica* (that causes CL worldwide) in India [77] and Bangladesh supported by subsequent molecular confirmation [31]. Similarly *L. tropica* causing VL has also been observed in US soldiers of Operation Desert Storm [78]. Also, *L. infantum*, and not *L. donovani* infection had been reported to have caused PKDL in a HIV-1 infected patient in Australia [79,80].

Experiments on clinical isolates from distinct atypical VL and CL endemic regions have identified strain-specific genetic variations upon sequence analysis of targeted genes, and polymorphisms of other regions defining parasite variants compared to the standard species-specific parasite genotypes associated with classical VL and/or CL disease phenotypes. Such new genetic variants can possibly explain the emergence of atypical leishmaniasis and thus the need for more studies on genetic analysis of the clinical isolates from known and newer disease foci for an insight into unusual phenotypic outcomes.

**Canine leishmaniasis**

VL in domestic dogs is another notable vector-borne zoonotic disease in humans. The causative organism is *L. infantum* and the disease is prevalent in Europe and South American countries [81]. Such VL-infested dogs/canines in these countries serve as a reservoir of VL. The key to the management of canine VL is continuous employment of prophylactic measures, through the correct use of repellents/insecticides and vaccines and prompt detection and monitoring of VL in dogs. In the middle East and in North Africa, canine CL due to *L. major* and *L. tropica* has been reported [82]. Also three beagle dogs displaying atypical VL due to *L. infantum* in Europe had been reported with rare granulomatous peritonitis [83]. Due to the importance of canine leishmaniasis as a natural reservoir for human disease, a comprehensive plan for its control including surveillance, phylogenetic studies and early and effective management should be employed to minimise its spread. There are several vaccines available to cure canine leishmaniasis, which exploit various antigens like LACK, A2, Q-protein, GP63, KMP-11, TYRP and etc. [84-89]. Challenged with such antigens provide protective immunity in the canines. Few commercialised vaccines for the canines are Leishmune, whose production and marketing licence had been withdrawn in 2014, Leish-Tec, LetiFend, and CaniLeish mostly



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

used in Brazil and European countries to treat dogs, although they do not work for humans [90-92].

**Other forms of leishmaniasis/parasites**

*Asymptomatic infections*

A significant challenge in the parasite elimination program is that a substantial proportion of healthy people living in endemic areas with no history of VL show positivity for antibodies to *Leishmania* owing to asymptomatic infections. Like PKDL, asymptomatic individuals are also considered as anthropogenetic reservoirs of VL. The guidelines of the panel of the American Society of Tropical Medicine and Hygiene (ASTMH) and Infectious Diseases Society of America (IDSA) suggest close monitoring of asymptomatic individuals with the initiation of treatment only upon symptom development [93]. Interestingly, these patients have elevated $CD4^+$ T cell counts and test positive for leishmanin skin test [94,95]. In addition, high level of IFNγ in $CD8^+$ T cells is also observed in such individuals with a few reported cases of elevated IL-17 and IL-22. [96]. These results suggest the protective role of host immune response against *Leishmania* infections and disease progression. Further focus on detection, understanding and tackling asymptomatic cases would be essential for effective development of strategies for elimination of leishmaniasis.

*Drug resistant parasites*

In the treatment of leishmaniasis, drug resistant strains (DRS) of *Leishmania* are a concerning issue. The emergence of DRS complicates the treatment efforts and underscores the need for ongoing research and development of new therapeutic strategies. *Leishmania* parasites exhibit genetic diversity, allowing some strains to develop resistance to specific drugs more easily than others. Pentavalent antimonial that became popular for use during the latter half of $20^{th}$ century has faced stiff resistance over the past decade or two, particularly in areas such as Bihar, India [97,98]. The increased antimonial unresponsiveness is ascribed to the inappropriate use of drug schedules, paving the way for progressive tolerance to drugs by the parasites [99]. The derivative of antimony, sodium stibogluconate (SSG) also has been discouraging due to the development of resistance to SSG by the parasite [100]. The genetically diverse Sb-resistant parasites displayed elevated thiol-synthesizing and antimony transporter gene expression compared to the susceptible ones [101]. As a lesson, antimonial are used in combination with



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

paromomycin as a first-line treatment for VL in East Africa to minimize the chance of resistance development [102,103].

Alternatively, lipid formulated amphotericin B deoxycholate is also being used against VL in ISC in place of just amphotericin B deoxycholate to reduce side effects. However, its high cost has become a major concern together with considerable number of relapses noticed in the ISC [103]. The other commonly known drug is miltefosine, an orally administered medication had been in use since 2002 in the ISC. However, resistance shown against miltefosine in VL patients has raised significant concerns in recent years [104]. Miltefosine's long half-life is responsible for retaining sub-therapeutic doses in circulation for an extended period, leading to exposure of surviving parasites to the drug for a longer period that is believed to result in the emergence of drug-resistance [46]. Apart from such pharmacokinetics-based reasons, there could also be parasites own mechanisms leading to resistance. In addition to such disadvantages its serious adverse side effects, which is believed to be due to immunopathological consequences has led to discontinuation of its use [104].

*Cohabitation with other animals/insects*The cohabitation of *Leishmania* parasites with other beings, including sandflies, reservoir hosts, and humans, as well as ecological and environmental factors, plays a crucial role in the transmission dynamics and epidemiology of leishmaniasis [1]. Zoonotically *Leishmania*-infected rodents or sand flies serve as reservoirs of infection for humans. Also, PKDL and atypical VL and CL patients serve as parasite reservoirs. The relationship between sand fly species and *Leishmania* can be complex and may vary depending on the region, ecology, and other factors. In the old world *Phlebotomus* spp. of sand flies transmit leishmaniasis, whereas in the new world *Lutzomyia* spp. are the vectors. Hence, the relationship between sand fly species and *Leishmania* can be complex and may vary depending on the region, ecology, and other factors. Majority of the cases of newly emergent foci of CL observed in recent years in the hilly regions of Himachal state in India have been reported along the Sutlej River belt. This could be due to the possible up stream migration of vectors along the rivers [105].



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

Cohabitation of *Leptomonas* with *Leishmania* has been much debated in recent years, especially when *L. donovani* and *Leptomonas seymouri,* which look alike were isolated in culture from VL patients [106-108]. Their high similarity results in the anomalous outcomes. Additionally, myosinXXI localization has been used as a biomarker to distinguish *Leptomonas* in *Leishmania* cultures [109]. To our knowledge, the involvement of *L. seymouri* in VL pathogenesis has not been assessed or reported in the literature. Furthermore, *Leptomonas* co-infection was also reported in a fraction of atypical CL cases caused by *L. donovani* in newer endemic pockets of Himachal Pradesh [110]. Also, identification of *L. seymouri* narna-like virus (NLV1) in serum samples of VL cases in India and its plausible role in disease progression has been reported [111,112], adding another dimension to the research on the causes of VL in the Indian subcontinent. Detection of *Leptomonas* spp. with a monoxenous life cycle and considered non-pathogenic to humans imply emerging evidence on the newer parasitic capability of this group of parasites. A rapid high-resolution melting based discriminatory diagnostic tool has been described to identify *Leptomonas* contamination in the VL clinical isolates [113], which can be used for further investigations.

*Comorbidity with other parasitic, bacterial and viral diseases*

Leishmaniasis frequently coexists with a range of other infections, including HIV, leprosy, tuberculosis, schistosomiasis, malaria, and more recently, COVID-19. These co-infections pose significant challenges due to the diverse pathological outcomes associated with varying host immune status. In many cases, co-infection exacerbates disease severity and increases mortality rates. Co-infection of VL with human immunodeficiency virus (HIV) is a life-threatening condition. This is because HIV infection and leishmaniasis together promote the replication of both causative pathogens and accelerates the progression of both VL and HIV [114,115]. The first reported case of VL/HIV coinfection in Europe was in 1980, and now it is documented in many countries, with the highest reports coming from Brazil, Ethiopia, and Bihar state in India. Patients coinfected with VL/HIV have the highest relapse rate and mortality, which poses significant challenges in the prevention and control of VL [116]. To address this issue, the WHO has recommended new guidelines to target VL in East Africa and South-East Asia based on the results of studies conducted in India by Médecins Sans Frontières and partners, and in Ethiopia by the Drugs for Neglected Diseases initiative and partners [117] HIV infected people contracting leishmaniasis are at a high risk of developing the full-blown disease and also high relapse and mortality rates [118,119]. Antiretroviral treatment is known to reduce the development of the disease, delay relapses and increase the survival rates. As of



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

2021, *Leishmania*-HIV coinfection has been reported in 45 countries. This has intensified the burden of leishmaniasis, due to the increased difficulty in clinical management and treatment of the disease.

Certainly, the interaction between leishmaniasis and COVID-19 co-infection is an emerging area of interest in the medical literature. According to a study published in 2020 [120], three cases of *Leishmania*-COVID-19 co-infection have been reported, highlighting the need for further investigation into the clinical implications of such co-occurrence. Another study [121] analyzed the clinical characteristics of *Leishmania*-SARS-CoV-2 co-infection and suggested that the presence of COVID-19 may lead to the reactivation of previously asymptomatic leishmaniasis. This finding underscores the importance of monitoring individuals with a history of *Leishmania* infection or their asymptomatics, particularly in regions where both diseases are endemic.

Interestingly, there is evidence suggesting a potential protective effect of *Leishmania* or other neglected tropical diseases against COVID-19 [120]. This observation may be attributed to the immune response mounted against *Leishmania* parasites, which could confer some level of immunity or resistance to SARS-CoV-2 infection. For example, the clearance of CL involves mast cells, cytotoxic CD8+ T cells, CD4+ helper T cells, and the production of IFN-γ [122,123], which are also important in controlling COVID-19. However, it is important to note that while an effective immune response is crucial in controlling both leishmaniasis and COVID-19, the timing and specific components of the immune response may vary between the two diseases. Early Th1 type of response is critical in controlling COVID-19, failure to do so can result in viral replication, tissue damage, and severe disease progression [124]. Further research is needed to elucidate the complex interactions between leishmaniasis and COVID-19 co-infection, including their impact on disease severity, immunopathogenesis, and treatment outcomes. This understanding will be essential for guiding clinical management and public health interventions in regions where both diseases are prevalent.

Co-infection of VL and tuberculosis (TB)/pulmonary tuberculosis (PTB) is common and a significant concern in regions, where both diseases are endemic, such as parts of Africa, Asia, and Latin America [125,126]. Second to VL, MCL also can co-exist with tuberculosis in certain parts of Asia [127,128].

Malaria, caused by the apicomplexan protozoan parasites *Plasmodium falciparum* or *P. vivax*, co-infecting with *Leishmania*, has been extensively reported worldwide [129]. For instance, a



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

case study from Malaysia documented a human infection with *P. vivax* (detected in a blood biofilm test) and Leishman-Donovan complex (involving *L. infantum* and *L. chagasi*) observed in bone marrow aspirate [130]. The prevalence of malaria co-infection with VL varies from 7-18% across different geographical areas in Asia and Africa. However, further longitudinal studies would be needed to fully understand their combined impact on the host and on each other.

Despite *Plasmodium* and *Leishmania* operating in different host cells and exhibiting distinct life cycles based on their unique biology and tropism, they may employ immune evasion strategies that commonly affect the host or increase the susceptibility to infections. This suggests a potential synergistic effect in co-infection scenarios, where the presence of one parasite could potentially modulate the host immune response, leading to increased susceptibility or severity of infection by the other parasite. Further research is needed to elucidate the precise mechanisms underlying these interactions and their implications for disease outcomes.

**Molecular typing and whole genome sequencing to study genotypic variations of *Leishmania* spp.**

The genus *Leishmania* encompasses a complex group of parasites with a wide range of genotypic (and phenotypic) characteristics, which are often used to divide them into species, subspecies and strains. Molecular tools developed in the field have made such classifications easy and relevant considering plastic nature of parasite genome with accumulation of newer genetic variations. Multilocus sequence typing (MST) [131,132], Randomly amplified polymorphic DNA (RAPD) [133,134]; Microsatellite typing (MT) [135,136], and Restriction fragment length polymorphism (RFLP) [137,138] are a few examples of such genotyping methods that addressed *Leishmania* variations.

In contrast to the widely used genotyping tools whole genome sequencing (WGS) provides detailed information on genetic variations across the entire genome, including single nucleotide polymorphisms (SNPs), insertions, deletions, and structural variations that may be more informative enabling studies on a range of aspects that include genetic diversity, polymorphisms, phylogeny, drug resistance and other disease aspects viz virulence factors, and



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

epidemiological surveillance. Complete/partial WGS information with nucleotide/gene/protein annotation information on several *Leishmania* species/strains are already available at https://tritrypdb.org/tritrypdb/app. Such species/strains of *Leishmania* include *L. aethiopica* L147, *L. amazonensis* MHOM/BR/71973/M2269, *L. amazonensis* strain PH8, *L. arabica* strain LEM1108, *L. braziliensis* MHOM/BR/75/M2903, *L. braziliensis* MHOM/BR/75/M2904, *L. braziliensis MHOM/BR/75/M2904 2019, L. donovani* BPK282A1, *L. donovani* CL-SL, *L. donovani* HU3, *L. donovani* strain LV9, *L. enriettii* MCAV/BR/2001/CUR178, *L. enriettii* strain LEM3045, *L. gerbilli* strain LEM452, *L. infantum* JPCM5, *L. major* Friedlin 2021, *L. major* strain LV39c5, *L. major* strain SD 75.1, *L. martiniquensis* LEM2494, *L. martiniquensis* MHOM/TH/2012/LSCM1, *L. mexicana* MHOM/GT/2001/U1103, *L. orientalis* MHOM/TH/2014/LSCM4, *L. panamensis* MHOM/COL/81/L13, *L. panamensis* strain MHOM/PA/94/PSC-1, *L.* sp. Ghana MHOM/GH/2012/GH5, *L.* sp. Namibia MPRO/NA/1975/252/LV425, *L. tarentolae* Parrot Tar II 2019, *L. tarentolae* Parrot-TarII, *L. tropica* L590, *Leishmania* turanica strain LEM423.

A recently described minicircle-based DNA footprint assay has simplified the detection and speciation of *Leishmania* clinical isolate [74]. This method has enabled the study of phylogenetic relationship and variations of many *Leishmania* species that have originated from different parts of the world. Parasites from CL lesions from red kangaroos of Australia [139] found to be grouped into unique cluster in the sequence based dendrogram analysis. This method enabled the detection of strain specific variations of *L. braziliensis* from Peru and Brazil that cause MCL [74]. Therefore, it remains as a promising approach for phylogenetic analysis, including measurement of the phylogenetic distances and identification of parasite isolates of unknown origin.

**Molecular basis of understanding parasite variants in leishmaniasis**

Several molecular tools have been developed in the past to investigate the evolutionary aspects and differentiate species or strains of *Leishmania*. Some of these tools, mentioned above, facilitate parasite groupings based on their genetic make-up in relation to phenotypic characteristics or clinical disease manifestations. These groupings may be crucial in detection of newer parasite variants circulating in different geographical sites and can help in aligning poilcy-makers for evidence driven strategies for disease diagnosis, treatment and elimination. Targeting multicopy DNA regions such as 18S rRNA, heat shock proteins, or mini- or



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

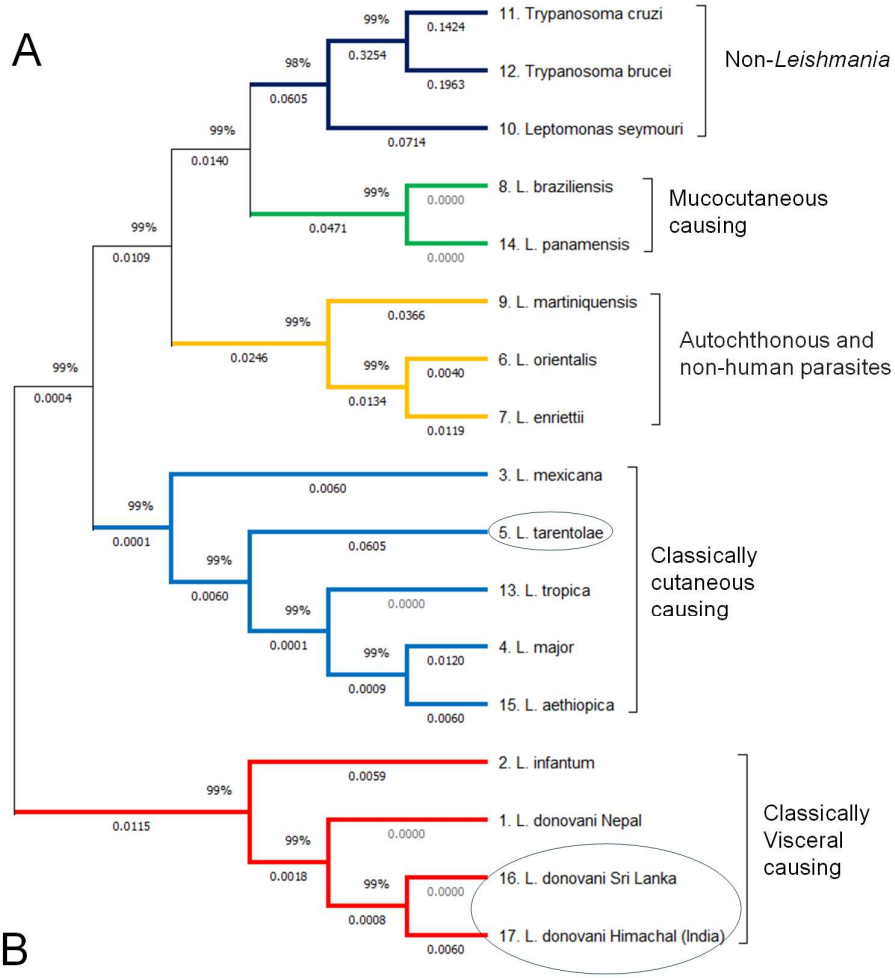
maxicircle kDNA regions for sequence-based analysis often poses challenges due to the huge heterogeneity of the said sequences in *Leishmania*. The sequence specific heterogeneity complicates the construction of accurate phylogenetic trees. Homologous recombination, gene conversion, and other evolutionary processes can obscure phylogenetic signals as a read out of species and strain identification. Addressing these challenges requires a multifaceted approach, combining appropriate molecular techniques, bioinformatics tools, and a thorough understanding of *Leishmania* biology. Towards this end, we attempted to develop a dendrogram-based analysis of a single-copy gene, centrin5 (calcium binding structural protein [140]), in *Leishmania* together with those of a few other Trypanosomatid parasites. The results are presented in **Fig. 2**. Among Trypanosomatid members, centrin5 proteins mostly consist of 165 amino acids. The tree analysis developed from 17 such centrin5 protein sequences of Trypanosomatid genera (comprising *Leishmania, Trypanosoma*, and *Leptomonas*) obtained through respective accession numbers (**Fig. 2** legend) displays distinct clades for typical VL, CL, and MCL parasites. There is a separate group having two autochthonous (*L. orientalis* and *L. martiniquensis*) [141] and one non-human parasite *L. enriettii* [142] (**Fig. 2A**). The three non-*Leishmania* parasites (*Trypanosoma brucei, Trypanosoma cruzi*, and *Leptomonas seymouri*) formed a separate group.

However, based on branch-length/subgrouping the outliers in some of these groups can be identified as distinct categories. For example, both the Sri Lankan and Himachal (India) *L. donovani* genetic variants that cause CL are seen closer to each other than classical VL causing *L. donovani* and *L. infantum* [143,144]. Their differences in amino acid percent identity are also compared in **Fig. 2B and C**. Also, interestingly, based on centrin5, *L. tarentolae*, the parasite that infects exclusively lizards [145] has been grouped with CL causing parasites (**Fig. 2A**). Overall, this study gives us a meaningful grouping of few of *Leishmania* and other genera of Trypanosomatid family that we attempted via cladogram analysis using MEGAX program. Such genetic variations across *Leishmania* species/strains circulating in known and newer endemic zones need molecular surveillance for detection and prediction of region-specific parasite variants and associated disease outcome.



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

A




B

```
L. donovani (Nepal)            MINTAFSTADGRRLPRDLTDQQKRDIEEAFRVLDVKGVNTITPNDLKVALRALGYEPDKD  60
L. infantum                    MINTAFSTADGRRLPRDLTDQQKRDIEEAFRVLDVKGVNTITPNDLKVALRALGYEPDKD  60
L. donovani (Sri Lanka)         MINTAFSTADGRRLPRDLTDQQKRDIEEAFRVLDVKGVNTITPNYLKVALRALGYEPDKD  60
L. donovani (Himachal, India) MINTAFSTADGRRLPRDLTDQQKRDIEEAFRVLDVKGVNTITPNDLKVALRALGYEPDKD  60
                               ****************************************** ***************

L. donovani (Nepal)            AVRKLVAEMDRGGVSSNLVLSEFEDVMRQRFFAEDRDDEVDLAFPLFTEGKSDFISLDDL 120
L. infantum                    AVRKLVAEMDRGGVSSNLVLSEFEDVMRQRFFAEDRDDEVDLAFPLFTEGKSDFISLDDL 120
L. donovani (Sri Lanka)         AVRKLVAEMDRGGVSSNLVLSEFEDVMRQRFFAEDRDDEVDLAFPLFTEGKSDFISLDDL 120
L. donovani (Himachal, India) AVRKLVAEMDRGGVSSNLVLSEFEDVMRQRFFAEDRDDEVDLAFPLFTEGKSDFISLDDL 120
                               ************************************************************

L. donovani (Nepal)            KRVAAEVGEDIPEDVLQEIIRECDVLDRDDRISREEFNKMLKSDK 165
L. infantum                    KRVAAEVGEDIPEDVLQEIIRECDVLDRDDRISREEFNKMLKSDK 165
L. donovani (Sri Lanka)         KRVAAEVGEDIPEDVLQEIIRECDVLDRDDRISREEFNKMLKSDK 165
L. donovani (Himachal, India) KRVAAEVGEDIPEDVLQEIIRECDVLDRDDRISREEFNRMLKSDK 165
                               ************************************:******
```

C

| *Leishmania* spp. | *L. donovani* (Nepal) | *L. infantum* | *L. donovani* (Sri Lanka) | *L. donovani* (Himachal, India) |
|---|---|---|---|---|
| *L. donovani* (Nepal) | 100.00 | | | |
| *L. infantum* | 99.39 | 100 | | |
| *L. donovani* (Sri Lanka) | 99.39 | 100 | 100.00 | |
| *L. donovani* (Himachal, India) | 98.79 | 99.39 | 99.38 | 100.00 |

**Figure 2: Molecular tool to support pathogenic variations.** A. Phylogenetic tree based on centrin5 proteins to infer group formations among *Leishmania* and other Trypanosome members. The parasite groups are described



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

by distinct colors and labelled at their right. The parasites genera and species are described with sources indicated for some. The accession numbers of centrin5 proteins of the parasites with their associated serial numbers include: 1. LdBPK_366370.1.1, 2. XP_001469992.1, 3. XP_003874970.1, 4. XP_001687199.1, 5. GET93710.1, 6. KAG5465198.1, 7. KAG5465937.1, 8. XP_001569255.1, 9. KAG5464459.1, 10. KPI90528.1, 11. KAF8287755.1, 12. XP_011778227.1, 13. LTRL590_360073600.1, 14. LPAL13_350071100.1, 15. LAEL147_000875700.1, 16. LdCL_360071100-t42_1, 17. AYU83995. The branch lengths and the bootstrap % values are also shown. The tree was constructed by Maximum Likelihood method and JTT matrix-based model using MEGA X program [146,147]. B & C. Multiple-sequence alignment and % identity of centrin5 proteins of only the 4 of the red branches 'A' above using Clustal W Omega program. The amino acid sequences of 17 centrin5 proteins, their combined Clustal W alignment and percent identity are described separately in the **Supplementary section**.

*Overall challenges, solutions, and conclusion*

Tropical countries are continuously striving to eliminate various forms of leishmaniasis endemic to their regions. A significant focus lies on combating VL, particularly in countries like India, where it poses a grave threat due to its potentially fatal nature. Challenges persist due to the persistent forms of PKDL and ALI, which hinder the progress of elimination programs. Continuous monitoring of cases through molecular screenings in endemic regions is essential to track occurrences effectively [148]. Preventing the development of drug resistance is a key aspect of elimination strategy. For pathogens that exhibit shifting clinical manifestations, such as atypical leishmaniasis, standard medications may prove ineffective. Hence, developing appropriate treatment regimens tailored to the evolving clinical nature of the disease becomes imperative in such cases.

In scenarios, where *Leishmania* species co-infect with other pathogens, such as viruses, bacteria, and parasites, they may collectively induce a synergistic immune response profile. This interaction can either enhance or limit the immune response, leading to decreased host resistance and a failure to control the infection [149]. However, it is important to note that each pathogen manipulates different aspects of the host immune response [150]. Therefore, the development of a broad-spectrum therapy against these infections could potentially eliminate not only the primary *Leishmania* infection but also any secondary and/or co-morbid infections. This approach would target a wide range of pathogens, providing a comprehensive treatment strategy to address the complexities of co-infection scenarios.

Disease prevention remains the cornerstone of sustainable leishmaniasis elimination efforts. Currently, for efficacy, the use of effective combinations of existing drugs is recommended for VL. For example, combinations such as miltefosine-AmBisome or miltefosine-paromomycin



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

559 have shown promise. These combinations also offer hope for co-infections. In Ethiopia,
560 AmBisome plus miltefosine has proven efficacious in HIV-VL patients. Additionally,
561 improved genetic, immunological, and serological markers are needed to determine the
562 progression from parasite infection to clinical VL. Markers for asymptomatic infections have
563 been utilized in clinical studies. However, in the absence of specific safe drugs or markers of
564 disease progression, further research is required to develop newer tools to address these
565 challenges. Various vaccine strategies have also been explored, including those utilizing
566 recombinant peptide, DNA, killed whole parasite, and genetically modified live-attenuated
567 parasites [151]. Notably, the *L. donovani* and *L. major* centrin gene knockout strains shows
568 promise as a live attenuated vaccine against both VL and CL [152-154]. Additionally, a clinical
569 trial utilizing ChAd63-KH, an adenoviral vaccine encoding KMP-11 and HASPB, was
570 conducted in Sudan with 24 PKDL patients, and the vaccine successfully generated a potent
571 innate and cell-mediated immune response [155]. Results showed that 30.4% of patients had
572 over 90% clinical improvement, while 21.7% showed partial improvement. Following this, a
573 Phase 2 vaccine trial with ChAd63-KH was conducted on 100 patients with persistent PKDL
574 in Sudan. This vaccine has been proven effective in those patients [156]. .
575
576 Periodic genome sequencing of the parasite isolates in affected regions can provide valuable
577 insights into emerging *Leishmania* variants. This data serves as an alert for clinicians and
578 researchers, prompting increased attention towards emerging parasite variants and the
579 associated clinical manifestation in region specific manner. Implementing effective measures
580 to control vector populations is another crucial approach for achieving successful elimination
581 of leishmaniasis. Addressing gaps in our understanding of vector bionomics is essential in this
582 regard. These gaps include screening for infected sand flies using PCR, determining sand fly
583 biting rates, assessing parasite infection rates within the vector population, and understanding
584 the spatial and temporal variations of these parameters in response to interventions such as
585 indoor residual spraying (IRS). Bridging these knowledge gaps is paramount to achieving
586 sustained elimination of VL and implementing an appropriate post-elimination program. Many
587 countries are now prioritizing boosting immunity to prevent infectious diseases, including
588 leishmaniasis, either as a primary infection or as an opportunistic infection alongside other
589 pathogens [52]. India's significant investment in AYUSH (Ayurveda, Yoga, Unani, Siddha,
590 and Homeopathy) as an alternative therapy approach exemplifies this direction. Other immune
591 modulators, such as liposomal cholesterol, which have proven effective experimentally in
592 treating VL, might need further studies. Some countries have transitioned to the post-



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

elimination maintenance phase for leishmaniasis control, emphasizing the importance of periodic screenings to detect any reemergence early and prevent resurgence. Although the march toward leishmaniasis elimination appears to be increasing, the achievement of the program remains uncertain in the light of already existing and newly emerging challenges like sporadic outbreaks, asymptomatic infections, and newer changing foci.

**Supplementary material.** The supplementary material for this article can be found**.**

**Funding statement.** AS is supported by Indian Council of Medical Research (ICMR), New Delhi, India [GIA/2/VBD/2021/ECD/II]. NDK is supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, USA under Award Number U01AI136033. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. MJ is supported by Indian Council of Medical Research, (ICMR), India [6/9-7 (272/KA/2021/ECD-II].

**Competing interests.** None.

## References

1. Selvapandiyan A, et al. (2019) Innovations for the elimination and control of visceral leishmaniasis. PLoS Negl Trop Dis 13: e0007616.

2. Rioux JA, et al. (1990) Taxonomy of Leishmania. Use of isoenzymes. Suggestions for a new classification. Ann Parasitol Hum Comp 65: 111-125.

3. Burza S, et al. (2018) Leishmaniasis. Lancet 392: 951-970.

4. Murray HW, et al. (2005) Advances in leishmaniasis. Lancet 366: 1561-1577.

5. Herwaldt BL (1999) Leishmaniasis. Lancet 354: 1191-1199.

6. Hailu T, et al. (2016) Challenges in visceral leishmaniasis control and elimination in the developing countries: A review. J Vector Borne Dis 53: 193-198.

7. Silva H, et al. (2021) Treatment failure to sodium stibogluconate in cutaneous leishmaniasis: A challenge to infection control and disease elimination. PLoS One 16: e0259009.

8. Handler MZ, et al. (2015) Cutaneous and mucocutaneous leishmaniasis: Clinical perspectives. J Am Acad Dermatol 73: 897-908; quiz 909-810.

9. Suqati AA, et al. (2020) Mucocutaneous leishmaniasis: case report and literature review of a rare endonasal infection. Pan Afr Med J 36: 292.

10. WHO (2020) Mucocutaneous Leishmaniasis. World Health Organization.

11. Bajgai GP, et al. (2023) Oral mucosal leishmaniasis presenting as a nonhealing chronic oral growth: A case report. Clin Case Rep 11: e7234.

12. Kumar A, et al. (2023) Post kala-azar dermal leishmaniasis in the Indian sub-continent: challenges and strategies for elimination. Front Immunol 14: 1236952.

13. Singh S, et al. (2011) Post-kala-azar dermal leishmaniasis: recent developments. Int J Dermatol 50: 1099-1108.

14. Kumar P, et al. (2021) Post Kala-Azar Dermal Leishmaniasis: Clinical Features and Differential Diagnosis. Indian J Dermatol 66: 24-33.

15. Zijlstra EE (2016) The immunology of post-kala-azar dermal leishmaniasis (PKDL). Parasit Vectors 9: 464.

16. Singh OP, et al. (2020) Xenodiagnosis to address key questions in visceral leishmaniasis control and elimination. PLoS Negl Trop Dis 14: e0008363.

17. Sampaio RNR, et al. (2021) Successful treatment of diffuse cutaneous leishmaniasis caused by Leishmania amazonensis. An Bras Dermatol 96: 602-604.

18. Kassardjian AA, et al. (2021) Diffuse cutaneous leishmaniasis and HIV co-infection: A case report and review of the literature. J Cutan Pathol 48: 802-806.

19. Badaro R, Johnson WD, Jr. (1993) The role of interferon-gamma in the treatment of visceral and diffuse cutaneous leishmaniasis. J Infect Dis 167 Suppl 1: S13-17.

20. Ul Bari A, Raza N (2010) Lupoid cutaneous leishmaniasis: a report of 16 cases. Indian J Dermatol Venereol Leprol 76: 85.

21. Pazoki H, et al. (2016) Lupoid leishmaniasis among the known cases of cutaneous leishmaniasis in Herat Province, western Afghanistan. J Infect Public Health 9: 557-563.

22. Khaled A, et al. (2011) Lupoid cutaneous leishmaniasis: a case report. Dermatol Ther (Heidelb) 1: 36-41.

23. Ali A, et al. (2006) Carbon dioxide laser for the treatment of lupoid cutaneous leishmaniasis (LCL): A case series of 24 patients. Dermatology Online Journal 12: 3.

24. Torres-Guerrero E, et al. (2017) Leishmaniasis: a review. F1000Res 6: 750.

25. Olliaro P, et al. (2018) Harmonized clinical trial methodologies for localized cutaneous leishmaniasis and potential for extensive network with capacities for clinical evaluation. PLoS Negl Trop Dis 12: e0006141.

26. Reithinger R, et al. (2007) Cutaneous leishmaniasis. Lancet Infect Dis 7: 581-596.

27. Kato H, et al. (2016) Geographic Distribution of Leishmania Species in Ecuador Based on the Cytochrome B Gene Sequence Analysis. PLoS Negl Trop Dis 10: e0004844.



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

664 28. Calvopina M, et al. (2006) Leishmania isoenzyme polymorphisms in Ecuador: relationships
665 with geographic distribution and clinical presentation. BMC Infect Dis 6: 139.

666 29. Calvopina M, et al. (2017) Coinfection of Leishmania guyanensis and Human
667 Immunodeficiency Virus-Acquired Immune Deficiency Syndrome: Report of a Case of
668 Disseminated Cutaneous Leishmaniasis in Ecuador. Am J Trop Med Hyg 96: 1151-
669 1154.

670 30. Machado GU, et al. (2019) Disseminated leishmaniasis: clinical, pathogenic, and
671 therapeutic aspects. An Bras Dermatol 94: 9-16.

672 31. Thakur L, et al. (2018) Atypical leishmaniasis: A global perspective with emphasis on the
673 Indian subcontinent. PLoS Negl Trop Dis 12: e0006659.

674 32. del Giudice P, et al. (1998) Cutaneous leishmaniasis due to Leishmania infantum. Case
675 reports and literature review. Arch Dermatol 134: 193-198.

676 33. Aoun K, Bouratbine A (2014) Cutaneous leishmaniasis in North Africa: a review. Parasite
677 21: 14.

678 34. Yadav P, et al. (2023) Unusual Observations in Leishmaniasis-An Overview. Pathogens
679 12.

680 35. Madeira MF, et al. (2006) Mixed infection with Leishmania (Viannia) braziliensis and
681 Leishmania (Leishmania) chagasi in a naturally infected dog from Rio de Janeiro,
682 Brazil. Trans R Soc Trop Med Hyg 100: 442-445.

683 36. Toz SO, et al. (2009) Leishmaniasis in Turkey: molecular characterization of Leishmania
684 from human and canine clinical samples. Trop Med Int Health 14: 1401-1406.

685 37. Najafi L, et al. (2021) Molecular and serological evaluation of zoonotic visceral
686 leishmaniasis in dogs in a rural area of Fars province, southern Iran, as a source of
687 Leishmania infantum infection. Vet Med Sci 7: 1082-1089.

688 38. Eiras DP, et al. (2015) Cutaneous Leishmaniasis: Current Treatment Practices in the USA
689 for Returning Travelers. Curr Treat Options Infect Dis 7: 52-62.

690 39. David CV, Craft N (2009) Cutaneous and mucocutaneous leishmaniasis. Dermatol Ther
691 22: 491-502.

692 40. WHO (2013) Leishmaniasis. https://wwwwhoint/news-room/fact-
693 sheets/detail/leishmaniasis: January 12.

694 41. Scorza BM, et al. (2017) Cutaneous Manifestations of Human and Murine Leishmaniasis.
695 Int J Mol Sci 18.

696 42. Sharma NL, et al. (2005) Localized cutaneous leishmaniasis due to Leishmania donovani
697 and Leishmania tropica: preliminary findings of the study of 161 new cases from a new
698 endemic focus in himachal pradesh, India. Am J Trop Med Hyg 72: 819-824.



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

699 43. Kumari A, et al. (2018) Diffuse Cutaneous Leishmaniasis in an Immunocompromised
700 Patient Resembling Histoid Hansen's Disease. Indian Dermatol Online J 9: 452-454.

701 44. Mehta V, et al. (2009) Diffuse cutaneous leishmaniasis in HIV. Dermatol Online J 15: 9.

702 45. Hashiguchi Y, et al. (2016) Diffuse and disseminated cutaneous leishmaniasis: clinical
703 cases experienced in Ecuador and a brief review. Trop Med Health 44: 2.

704 46. Guimaraes LH, et al. (2009) Atypical manifestations of tegumentary leishmaniasis in a
705 transmission area of Leishmania braziliensis in the state of Bahia, Brazil. Trans R Soc
706 Trop Med Hyg 103: 712-715.

707 47. Oliveira-Neto MP, et al. (1998) Leishmaniasis recidiva cutis in New World cutaneous
708 leishmaniasis. Int J Dermatol 37: 846-849.

709 48. Tejura N, et al. (2019) Case Report: Mucocutaneous Leishmaniasis Masquerading as
710 Idiopathic Midline Granulomatous Disease. Am J Trop Med Hyg 101: 1107-1110.

711 49. Scarpini S, et al. (2022) Visceral Leishmaniasis: Epidemiology, Diagnosis, and Treatment
712 Regimens in Different Geographical Areas with a Focus on Pediatrics. Microorganisms
713 10.

714 50. Rakhshanpour A, et al. (2014) Serological Survey and Associated Risk Factors of Visceral
715 Leish-maniasis in Qom Province, Central Iran. Iran J Public Health 43: 50-55.

716 51. Patil RR, Chatterjee PK (2023) Epidemiology of Visceral Leishmaniasis in India. FROM
717 THE EDITED VOLUME *Leishmania* Parasites - Epidemiology, Immunopathology
718 and Hosts by Edited by Almeida-Souza, F de Oliveira Cardoso, F de O Abreu-Silva AL
719 and Calabrese, KDS.

720 52. Farooq I, et al. (2023) The Burden of Visceral Leishmaniasis: Need of Review, Innovations,
721 and Solutions. A Selvapandiyan et a (eds) Challenges and Solutions Against Visceral
722 Leishmaniasis Springer Nature Singapore Pte Ltd: 1-17.

723 53. WHO (2021) Visceral leishmaniasis elimination: India gears-up to overcome last-mile
724 challenges. https://wwwwhoint/japan/news/detail-global/29-07-2021-visceral-
725 leishmaniasis-elimination-india-gears-up-to-overcome-last-mile-challenges: 29 July.

726 54. Pilot E, et al. (2019) The Organization, Implementation, and Functioning of Dengue
727 Surveillance in India-A Systematic Scoping Review. Int J Environ Res Public Health
728 16.

729 55. WHO (2019) Independent assessment of Kala-Azar elimination programme in India.
730 https://wwwwhoint/publications/i/item/9789290227960: 9-20 December.

731 56. Saha P, et al. (2021) Sir U.N. Brahmachari and his battle against Kala-Azar. Trop Parasitol
732 11: 89-91.



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

733 57. Joshi S, et al. (2021) Rapid Multiplex Loop-Mediated Isothermal Amplification (m-LAMP)
734 Assay for Differential Diagnosis of Leprosy and Post-Kala-Azar Dermal
735 Leishmaniasis. Am J Trop Med Hyg 104: 2085-2090.

736 58. Bhandari V, et al. (2012) Drug susceptibility in Leishmania isolates following miltefosine
737 treatment in cases of visceral leishmaniasis and post kala-azar dermal leishmaniasis.
738 PLoS Negl Trop Dis 6: e1657.

739 59. Ramesh V, et al. (2020) Assessing the Efficacy and Safety of Liposomal Amphotericin B
740 and Miltefosine in Combination for Treatment of Post Kala-Azar Dermal
741 Leishmaniasis. J Infect Dis 221: 608-617.

742 60. Sarraf NR, et al. (2021) Genome wide comparison of Leishmania donovani strains from
743 Indian visceral leishmaniasis and para-kala-azar dermal leishmaniasis patients. Acta
744 Trop 223: 106086.

745 61. Maruf S, et al. (2023) Revisiting the diagnosis and treatment of Para Kala-azar Dermal
746 Leishmaniasis in the endemic foci of Bangladesh. PLoS One 18: e0280747.

747 62. Kumar R, et al. (2016) Para-kala-azar dermal Leishmaniasis cases in Indian subcontinent -
748 A case series. Pathog Glob Health 110: 326-329.

749 63. Lindoso JAL, et al. (2018) Para-kala-azar dermal leishmaniasis in a patient in Brazil: a case
750 report. Rev Soc Bras Med Trop 51: 105-107.

751 64. Alves NO, et al. (2024) VL-HIV co-infection with Leishmania containing skin lesions
752 resembling para-kala-azar dermal leishmaniasis. PLoS Negl Trop Dis 18: e0012438.

753 65. Karunaweera ND (2009) Leishmania donovani causing cutaneous leishmaniasis in Sri
754 Lanka: a wolf in sheep's clothing? Trends Parasitol 25: 458-463.

755 66. Siriwardana Y, et al. (2019) Leishmania donovani Induced Cutaneous Leishmaniasis: An
756 Insight into Atypical Clinical Variants in Sri Lanka. J Trop Med 2019: 4538597.

757 67. Samarasinghe SR, et al. (2018) Genomic insights into virulence mechanisms of Leishmania
758 donovani: evidence from an atypical strain. BMC Genomics 19: 843.

759 68. Lypaczewski P, Matlashewski G (2021) Leishmania donovani hybridisation and
760 introgression in nature: a comparative genomic investigation. Lancet Microbe 2: e250-
761 e258.

762 69. Silva H, et al. (2024) Autochthonous Leishmaniasis Caused by Leishmania tropica,
763 Identified by Using Whole-Genome Sequencing, Sri Lanka. Emerg Infect Dis 30: 1872-
764 1883.

765 70. Lypaczewski P, et al. (2022) An intraspecies Leishmania donovani hybrid from the Indian
766 subcontinent is associated with an atypical phenotype of cutaneous disease. iScience
767 25: 103802.



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

768 71. Pradhan A, et al. (2020) Atypical Presentation of Post-Kala-Azar Dermal Leishmaniasis in
769 Bhutan. Case Rep Dermatol Med 2020: 8899586.

770 72. Rai T, et al. (2023) Leishmania donovani persistence and circulation causing cutaneous
771 leishmaniasis in unusual-foci of Nepal. Sci Rep 13: 12329.

772 73. Kariyawasam UL, et al. (2017) Genetic diversity of Leishmania donovani that causes
773 cutaneous leishmaniasis in Sri Lanka: a cross sectional study with regional
774 comparisons. BMC Infect Dis 17: 791.

775 74. Selvapandiyan A, et al. (2008) A Leishmania minicircle DNA footprint assay for sensitive
776 detection and rapid speciation of clinical isolates. Transfusion 48: 1787-1798.

777 75. Pulimood SA, et al. (2012) Atypical mucocutaneous involvement with Leishmania
778 donovani. Natl Med J India 25: 148-150.

779 76. Sethuraman G, et al. (2008) Indian mucosal leishmaniasis due to Leishmania donovani
780 infection. N Engl J Med 358: 313-315.

781 77. Sacks DL, et al. (1995) Indian kala-azar caused by Leishmania tropica. Lancet 345: 959-
782 961.

783 78. Magill AJ, et al. (1993) Visceral infection caused by Leishmania tropica in veterans of
784 Operation Desert Storm. N Engl J Med 328: 1383-1387.

785 79. Stark D, et al. (2006) Post-kala-azar dermal leishmaniasis due to Leishmania infantum in a
786 human immunodeficiency virus type 1-infected patient. J Clin Microbiol 44: 1178-
787 1180.

788 80. Carnauba D, Jr., et al. (2009) Atypical disseminated leishmaniasis similar to post-kala-azar
789 dermal leishmaniasis in a Brazilian AIDS patient infected with Leishmania
790 (Leishmania) infantum chagasi: a case report. Int J Infect Dis 13: e504-507.

791 81. Almeida M, et al. (2022) Seroprevalence and Risk Factors Associated with Leishmania
792 Infection in Dogs from Portugal. Microorganisms 10.

793 82. Baneth G, et al. (2017) Canine leishmaniosis caused by Leishmania major and Leishmania
794 tropica: comparative findings and serology. Parasit Vectors 10: 113.

795 83. Peris MP, et al. (2022) Atypical Lesions in Canine Leishmaniosis: Description of New
796 Cases. Animals (Basel) 12.

797 84. Sinha S, et al. (2013) A comprehensive analysis of LACK (Leishmania homologue of
798 receptors for activated C kinase) in the context of Visceral Leishmaniasis.
799 Bioinformation 9: 832-837.

800 85. Coelho EA, et al. (2003) Immune responses induced by the Leishmania (Leishmania)
801 donovani A2 antigen, but not by the LACK antigen, are protective against experimental
802 Leishmania (Leishmania) amazonensis infection. Infect Immun 71: 3988-3994.



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

803 86. Chan A, et al. (2021) The role of Leishmania GP63 in the modulation of innate
804 inflammatory response to Leishmania major infection. PLoS One 16: e0262158.

805 87. Carson C, et al. (2009) A prime/boost DNA/Modified vaccinia virus Ankara vaccine
806 expressing recombinant Leishmania DNA encoding TRYP is safe and immunogenic in
807 outbred dogs, the reservoir of zoonotic visceral leishmaniasis. Vaccine 27: 1080-1086.

808 88. Basu R, et al. (2005) Kinetoplastid membrane protein-11 DNA vaccination induces
809 complete protection against both pentavalent antimonial-sensitive and -resistant strains
810 of Leishmania donovani that correlates with inducible nitric oxide synthase activity and
811 IL-4 generation: evidence for mixed Th1- and Th2-like responses in visceral
812 leishmaniasis. J Immunol 174: 7160-7171.

813 89. Carcelen J, et al. (2009) The chimerical multi-component Q protein from Leishmania in
814 the absence of adjuvant protects dogs against an experimental Leishmania infantum
815 infection. Vaccine 27: 5964-5973.

816 90. Velez R, Gallego M (2020) Commercially approved vaccines for canine leishmaniosis: a
817 review of available data on their safety and efficacy. Trop Med Int Health 25: 540-557.

818 91. Shital S, et al. (2024) An update on recombinant vaccines against leishmaniasis. Indian J
819 Med Res 160: 323-337.

820 92. Selvapandiyan A, et al. (2024) Worldwide Efforts for the Prevention of Visceral
821 Leishmaniasis Using Vaccinations. Book Springer Nature, Singapore Editors: : 413-
822 426.

823 93. Ready PD (2014) Epidemiology of visceral leishmaniasis. Clin Epidemiol 6: 147-154.

824 94. Hailu A, et al. (2005) T cell subset and cytokine profiles in human visceral leishmaniasis
825 during active and asymptomatic or sub-clinical infection with Leishmania donovani.
826 Clin Immunol 117: 182-191.

827 95. Mary C, et al. (1999) Control of Leishmania infantum infection is associated with CD8(+)
828 and gamma interferon- and interleukin-5-producing CD4(+) antigen-specific T cells.
829 Infect Immun 67: 5559-5566.

830 96. Pitta MG, et al. (2009) IL-17 and IL-22 are associated with protection against human kala
831 azar caused by Leishmania donovani. J Clin Invest 119: 2379-2387.

832 97. Ponte-Sucre A, et al. (2017) Drug resistance and treatment failure in leishmaniasis: A 21st
833 century challenge. PLoS Negl Trop Dis 11: e0006052.

834 98. Madusanka RK, et al. (2024) Antimony resistance and gene expression in Leishmania:
835 spotlight on molecular and proteomic aspects. Parasitology 151: 1-14.

836 99. Sundar S, et al. (1994) Clinicoepidemiological study of drug resistance in Indian kala-azar.
837 BMJ 308: 307.



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

838 100. Carter KC, et al. (2005) Sodium stibogluconate resistance in Leishmania donovani
839 correlates with greater tolerance to macrophage antileishmanial responses and trivalent
840 antimony therapy. Parasitology 131: 747-757.

841 101. Khanra S, et al. (2022) Antimony resistance mechanism in genetically different clinical
842 isolates of Indian Kala-azar patients. Front Cell Infect Microbiol 12: 1021464.

843 102. WHO (2010) Control of the leishmaniasis: report of a meeting of the WHO Expert
844 Committee on the Control of Leishmaniases, Geneva, 22-26 March 2010. 949 949.

845 103. Sundar S, Singh A (2018) Chemotherapeutics of visceral leishmaniasis: present and future
846 developments. Parasitology 145: 481-489.

847 104. Singh R, et al. (2023) Miltefosine Unresponsiveness in Visceral Leishmaniasis. A
848 Selvapandiyan et a (eds) Challenges and Solutions Against Visceral Leishmaniasis
849 Springer Nature Singapore Pte Ltd.

850 105. Sharma A, et al. (2023) Emerging leishmaniasis in southern Himalayas: A mini-
851 review. World Journal of Clinical Infectious Diseases 13: 11-23.

852 106. Ahuja K, et al. (2015) Selective elimination of Leptomonas from the in vitro co-culture
853 with Leishmania. Parasitol Int 64: 1-5.

854 107. Selvapandiyan A, et al. (2015) Implications of co-infection of Leptomonas in visceral
855 leishmaniasis in India. Parasitology 142: 1657-1662.

856 108. Srivastava P, et al. (2010) Detection of Leptomonas sp. parasites in clinical isolates of
857 Kala-azar patients from India. Infect Genet Evol 10: 1145-1150.

858 109. Kajuluri LP, et al. (2022) Intracellular localization of MyosinXXI discriminates
859 Leishmania spp and Leptomonasseymouri. Biochem Biophys Res Commun 604: 70-
860 75.

861 110. Thakur L, et al. (2020) Leptomonas seymouri Co-infection in Cutaneous Leishmaniasis
862 Cases Caused by Leishmania donovani From Himachal Pradesh, India. Front Cell
863 Infect Microbiol 10: 345.

864 111. Sukla S, et al. (2022) Detection of Leptomonas seymouri narna-like virus in serum
865 samples of visceral leishmaniasis patients and its possible role in disease pathogenesis.
866 Sci Rep 12: 14436.

867 112. Sukla S, et al. (2017) Leptomonas seymouri narna-like virus 1 and not leishmaniaviruses
868 detected in kala-azar samples from India. Arch Virol 162: 3827-3835.

869 113. Ahuja K, et al. (2020) High resolution melting based method for rapid discriminatory
870 diagnosis of co-infecting Leptomonas seymouri in Leishmania donovani-induced
871 leishmaniasis. Parasitol Int 75: 102047.

872 114. Tremblay M, et al. (1996) Leishmania and the pathogenesis of HIV infection. Parasitol
873 Today 12: 257-261.



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

874 115. Mock DJ, et al. (2012) Leishmania induces survival, proliferation and elevated cellular
875 dNTP levels in human monocytes promoting acceleration of HIV co-infection. PLoS
876 Pathog 8: e1002635.

877 116. Takele Y, et al. (2022) Immunological factors, but not clinical features, predict visceral
878 leishmaniasis relapse in patients co-infected with HIV. Cell Rep Med 3: 100487.

879 117. WHO (2022) WHO guideline for the treatment of visceral leishmaniasis in HIV co-
880 infected patients in East Africa and South-East Asia
881 https://wwwwhoint/publications/i/item/9789240048294: 7 June.

882 118. Diro E, et al. (2019) A randomized trial of AmBisome monotherapy and AmBisome and
883 miltefosine combination to treat visceral leishmaniasis in HIV co-infected patients in
884 Ethiopia. PLoS Negl Trop Dis 13: e0006988.

885 119. Rahman R, et al. (2017) Safety and efficacy of short course combination regimens with
886 AmBisome, miltefosine and paromomycin for the treatment of visceral leishmaniasis
887 (VL) in Bangladesh. PLoS Negl Trop Dis 11: e0005635.

888 120. Saidi N, Jelassi R (2023) Does infection with Leishmania protect against Covid-19?
889 Immunol Lett 253: 28-29.

890 121. Pikoulas A, et al. (2022) Visceral leishmaniasis and COVID-19 coinfection - A case
891 report. IDCases 27: e01358.

892 122. Muller AJ, et al. (2012) CD4+ T cells rely on a cytokine gradient to control intracellular
893 pathogens beyond sites of antigen presentation. Immunity 37: 147-157.

894 123. Naqvi N, et al. (2020) Host Mast Cells in Leishmaniasis: Friend or Foe? Trends Parasitol
895 36: 952-956.

896 124. De Biasi S, et al. (2020) Marked T cell activation, senescence, exhaustion and skewing
897 towards TH17 in patients with COVID-19 pneumonia. Nat Commun 11: 3434.

898 125. Gautam S, et al. (2014) CD8 T cell exhaustion in human visceral leishmaniasis. J Infect
899 Dis 209: 290-299.

900 126. Li XX, Zhou XN (2013) Co-infection of tuberculosis and parasitic diseases in humans: a
901 systematic review. Parasit Vectors 6: 79.

902 127. Rathnayake D, et al. (2010) Co-infection of mucosal leishmaniasis and extra pulmonary
903 tuberculosis in a patient with inherent immune deficiency. Int J Dermatol 49: 549-551.

904 128. Strazzulla A, et al. (2013) Mucosal leishmaniasis: an underestimated presentation of a
905 neglected disease. Biomed Res Int 2013: 805108.

906 129. Ornellas-Garcia U, et al. (2023) Malaria and leishmaniasis: Updates on co-infection. Front
907 Immunol 14: 1122411.



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

908 130. Ab Rahman AK, Abdullah FH (2011) Visceral leishmaniasis (kala-azar) and malaria
909 coinfection in an immigrant in the state of Terengganu, Malaysia: A case report. J
910 Microbiol Immunol Infect 44: 72-76.

911 131. El Mazini S, et al. (2023) Genetic Diversity and Population Structure of Leishmania
912 infantum in Morocco as Revealed by Multilocus Sequence Typing (MLST) Approach.
913 Pathogens 12.

914 132. Banu SS, et al. (2019) A novel multilocus sequence typing scheme identifying genetic
915 diversity amongst Leishmania donovani isolates from a genetically homogeneous
916 population in the Indian subcontinent. Int J Parasitol 49: 555-567.

917 133. Ikram G, et al. (2002) Random amplified polymorphic DNA technique for identification
918 and differentiation of old world Leishmania species. Am J Trop Med Hyg 66: 152-156.

919 134. Yazidi R, et al. (2015) RAPD-PCR reveals genetic polymorphism among Leishmania
920 major strains from Tunisian patients. BMC Infect Dis 15: 269.

921 135. Karakus M, et al. (2019) The impact of refugees on leishmaniasis in Turkey: a new
922 Syrian/Turkish Leishmania tropica population structure described by multilocus
923 microsatellite typing (MLMT). Parasitol Res 118: 2679-2687.

924 136. Rugna G, et al. (2018) Multilocus microsatellite typing (MLMT) reveals host-related
925 population structure in Leishmania infantum from northeastern Italy. PLoS Negl Trop
926 Dis 12: e0006595.

927 137. Usmael UA, et al. (2022) Detection of Leishmania donovani using ITS1-RFLP from
928 positive and negative smear samples among clinically reported patients visiting
929 University of Gondar Comprehensive Specialized Hospital. BMC Infect Dis 22: 963.

930 138. Koohsar F, et al. (2020) Molecular identification of Leishmania species isolated from
931 patients with cutaneous leishmaniosis in gonbad Kavoos, northeastern of Iran using
932 hSP70 and ITS-based PCR-RFlP. Ann Parasitol 66: 339-346.

933 139. Rose K, et al. (2004) Cutaneous leishmaniasis in red kangaroos: isolation and
934 characterisation of the causative organisms. Int J Parasitol 34: 655-664.

935 140. Selvapandiyan A, et al. (2007) Centrin1 is required for organelle segregation and
936 cytokinesis in Trypanosoma brucei. Mol Biol Cell 18: 3290-3301.

937 141. Anuntasomboon P, et al. (2022) Comparative Draft Genomes of Leishmania orientalis
938 Isolate PCM2 (Formerly Named Leishmania siamensis) and Leishmania martiniquensis
939 Isolate PCM3 from the Southern Province of Thailand. Biology (Basel) 11.

940 142. Paranaiba LF, et al. (2017) Leishmania enriettii (Muniz & Medina, 1948): A highly
941 diverse parasite is here to stay. PLoS Pathog 13: e1006303.

942 143. Siriwardana HV, et al. (2007) Leishmania donovani and cutaneous leishmaniasis, Sri
943 Lanka. Emerg Infect Dis 13: 476-478.



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press

144. Thakur L, et al. (2020) Leishmania donovani Infection with Atypical Cutaneous Manifestations, Himachal Pradesh, India, 2014-2018. Emerg Infect Dis 26: 1864-1869.

145. Raymond F, et al. (2012) Genome sequencing of the lizard parasite Leishmania tarentolae reveals loss of genes associated to the intracellular stage of human pathogenic species. Nucleic Acids Res 40: 1131-1147.

146. Kumar S, et al. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol 35: 1547-1549.

147. Ahmad S, et al. (2000) Phylogenetic analysis of gram-positive bacteria based on grpE, encoded by the dnaK operon. Int J Syst Evol Microbiol 50 Pt 5: 1761-1766.

148. Selvapandiyan A, et al. (2005) A novel semiquantitative fluorescence-based multiplex polymerase chain reaction assay for rapid simultaneous detection of bacterial and parasitic pathogens from blood. J Mol Diagn 7: 268-275.

149. Supali T, et al. (2010) Polyparasitism and its impact on the immune system. Int J Parasitol 40: 1171-1176.

150. Selvapandiyan A, et al. (2023) Zooming in on common immune evasion mechanisms of pathogens in phagolysosomes: potential broad-spectrum therapeutic targets against infectious diseases. FEMS Microbiol Rev 47.

151. Selvapandiyan A, et al. (2023) Worldwide Efforts for the Prevention of Visceral Leishmaniasis Using Vaccinations A Selvapandiyan et a (eds) Challenges and Solutions Against Visceral Leishmaniasis Springer Nature Singapore Pte Ltd: 413-426.

152. Avishek K, et al. (2016) Gene deleted live attenuated Leishmania vaccine candidates against visceral leishmaniasis elicit pro-inflammatory cytokines response in human PBMCs. Sci Rep 6: 33059.

153. Bhattacharya P, et al. (2016) Live Attenuated Leishmania donovani Centrin Knock Out Parasites Generate Non-inferior Protective Immune Response in Aged Mice against Visceral Leishmaniasis. PLoS Negl Trop Dis 10: e0004963.

154. Zhang WW, et al. (2020) A second generation leishmanization vaccine with a markerless attenuated Leishmania major strain using CRISPR gene editing. Nat Commun 11: 3461.

155. Younis BM, et al. (2021) Safety and immunogenicity of ChAd63-KH vaccine in post-kala-azar dermal leishmaniasis patients in Sudan. Mol Ther 29: 2366-2377.

156. Lacey C, et al. (2022) LEISH2b - A phase 2b study to assess the safety, efficacy, and immunogenicity of the Leishmania vaccine ChAd63-KH in post-kala azar dermal leishmaniasis. Wellcome Open Res 7: 200.



https://doi.org/10.1017/erm.2025.4 Published online by Cambridge University Press