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An Update on Clinical and Pathogenic Spectra of Leishmaniasis

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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 the development of molecular surveillance tools to detect 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 facilitates the search for and the design of targeted interventions. In this context, this review will navigate through the dynamic terrain of leishmaniasis, the various causative species of *Leishmania* parasites emphasising the urgency for the development of robust surveillance mechanisms and innovative approaches to confront the evolving challenges in our quest for global disease elimination.

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 (Ref. 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 (KA)). 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 (Ref. 2). The order Kinetoplastida is a group of single-celled parasitic protozoa characterised 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.

Cutaneous leishmaniasis (typical)

CL, the most prevalent form of the disease, is characterised 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*

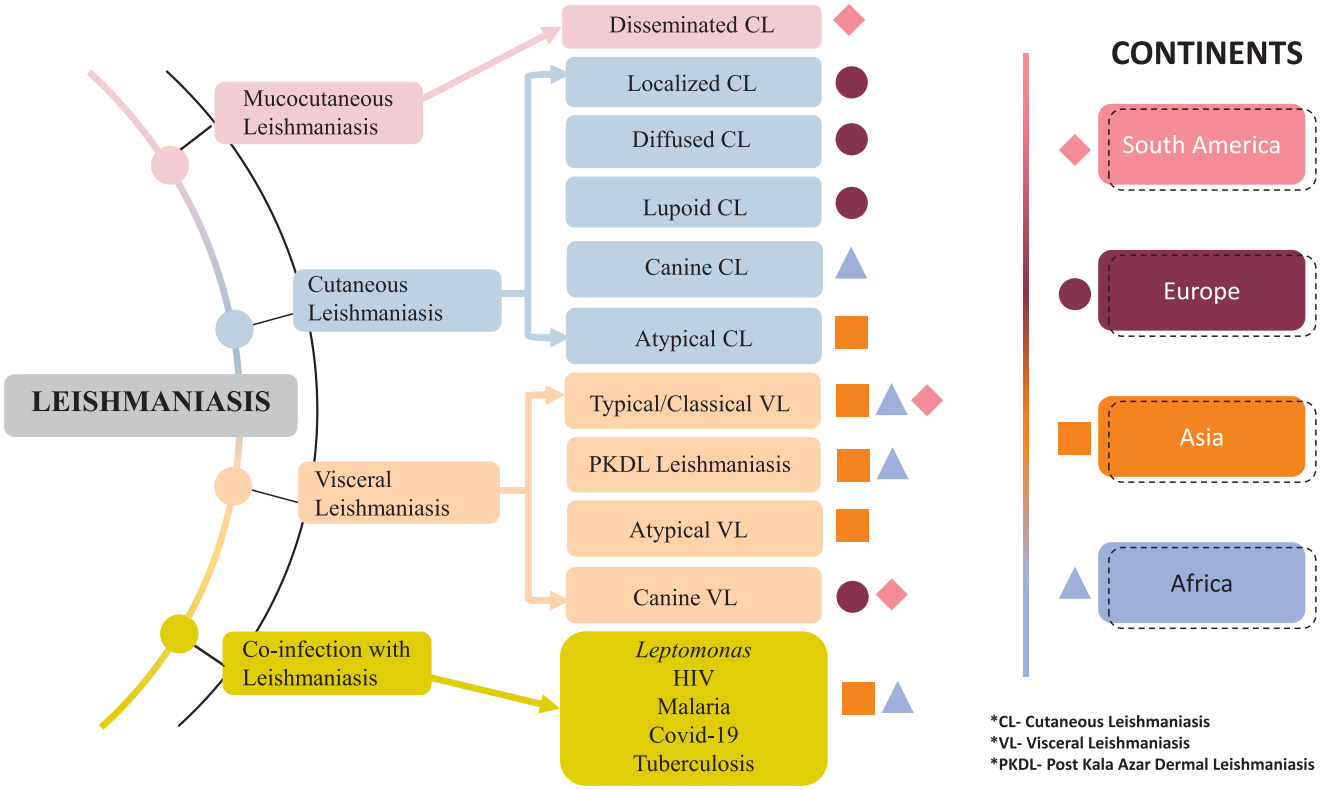

Figure 1. The sketch describes various forms of leishmaniases and the continents where each of the diseases is prevalent.

and *L. infantum* complexes in the Old World (Ref. 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 6 months without intervention. The specific ulcerating granuloma of skin is an initial papule, later transforming into an ulcer (Ref. 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 (Ref. 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 the potential for significant public health impacts, emphasising 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 (Ref. 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 outcomes are discussed in the 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 (Ref. 42). The affected body sites are the ears, nose, upper lip, cheeks, legs, hands and forearms.

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

Diffuse cutaneous leishmaniasis

In rare incidences of CL, diffuse cutaneous leishmaniasis (DCL) may arise, characterised by non-ulcerating nodules affecting large skin areas, causing prolonged and severe disabilities persisting for months or even years (Ref. 43). This is characterised by an anergic of cellular immune response to parasite antigens (Ref. 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 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 parasitised macrophages in DCL (Ref. 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.

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

S. no.	Type of leishmaniasis	*Leishmania* sp.	Regions affected	Clinical symptoms	Challenges in eradication
1	Visceral	*L. donovani*, *L. infantum*, *L. chagasi*	Bangladesh, Brazil, India, Ethiopia, Sudan, Nepal and South Sudan (Ref. 3)	Fever, weight loss, organomegaly, lymphadenopathy, anaemia, thrombocytopenia, neutropenia, hyperglobulinaemia, hepatic dysfunction, jaundice and ascites (Refs 4, 5)	Poor vector control strategies, limited diagnostic services, inadequate drug availability, limited treatment options and a lack of community awareness (Ref. 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, the Middle East and Central Asia (Ref. 3)	Ulcerating lesions, single or multiple, palpable lymph nodes (Ref. 3)	Treatment failure due to various factors such as age, number and size of lesions (Ref. 7).
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* (Ref. 8)	Germany, Central and South America (Refs 9, 10)	Nasal secretions, nasal obstruction, pain, epistaxis. Destructive lesions in the nose, oropharynx. Initially involves the nose and mouth, can progress to the pharynx and larynx (Ref. 8)	Diagnosis of oral leishmaniasis is a challenge mainly because it is rarely encountered (Ref. 11), primarily due to the lack of a laboratory setting.
4	Post-Kala-Azar Dermal	*L. donovani*, *L. infantum*, *L. tropica* (Refs 12, 13)	India, Sudan, South Sudan, Bangladesh, Ethiopia, Nepal, Mediterranean countries and Latin America (Refs 12, 13)	Hypopigmented patches, erythematous succulent papulo-plaques and nodular lesions on the face, upper body, genitalia and tongue. Atypical symptoms – photosensitivity, verrucous, hypertrophic, xanthomatous and ulcerative lesions (Ref. 14)	Unseeking treatment as symptoms are minimal. Thus, it acts as a reservoir (Refs 15, 16).
5	Diffuse cutaneous	*L. amazonensis* (Ref. 17)	Brazil (Ref. 17)	Diffuse papulonodular eruption on trunk and upper extremities (Ref. 18)	Resistant to chemotherapy and is also associated with an absent cell-mediated response (Ref. 19).
6	Lupoid	*L. tropica*	Pakistan (Ref. 20), Afghanistan, Africa and Europe (Ref. 21)	Erythematous and infiltrated plaque, psoriasiform (Ref. 22), ulcerated/crusted and discoid lupus erythematosus-like, solitary plaque extending over the face and multiple lesions (Ref. 20)	Microscopic examination of direct smears has low sensitivity for diagnosis. CO_2 laser radiation-based treatment seems efficient for LCL (Ref. 23).
7	Localised cutaneous	*L. mexicana*, *L. tropica* (Ref. 24)	Afghanistan, Nicaragua, Algeria, Brazil, Syrian Arab Republic, Honduras, Iran, Pakistan, Peru, Colombia, Saudi Arabia, Tunisia, Turkey, Morocco and Yemen (Ref. 25)	Single papular or nodular skin lesion that progressively ulcerates (Ref. 26)	Severe pain due to papular or nodular skin lesions, due to delay in diagnosis and treatment, is a major challenge.
8	Disseminated	*L. panamensis*, *L. guyanensis*	Subtropical and tropical lowlands of the Pacific coastal region (Refs 27, 28)	Disseminated pleomorphic ulcers, papules and cutaneous plaque-like lesions (Ref. 29)	DCL has been poorly studied. The failure rate of pentavalent antimony therapy is 75% (Ref. 30).
9	Atypical	*CL-L. donovani complex*, *L. infantum*, *L. chagasi*, VL-*L. tropica complex*, *L. amazonensis* and *L. major* (Ref. 31)	Mediterranean Region, North Africa, Europe and America (Ref. 32) Bahia, Brazil, Saudi Arabia, Iran, Kenya, Israel and India (Ref. 33)	Same as typical VL. 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 (Ref. 34).

(*Continued*)

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Table 1. (*Continued*)

S. no.	Type of leishmaniasis	*Leishmania* sp.	Regions affected	Clinical symptoms	Challenges in eradication
10	Canine	*L. (V) braziliensis*, *L. (V) chagasi*, *L (V.) panamensis*, *L (V.) peruviana*, *L (V.) guyanensis* (Ref. 35), *L. infantum* (Ref. 36)	Marmara, Ege, Black Sea and Mediterranean regions of western Turkey (Ref. 36)	Local, ulcerative lesions on the nipples, scrotum, ears, feet and muzzle (Ref. 35)	Canine leishmaniasis serves as a reservoir host of VL (Refs 1, 37).

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 (Ref. 30). In almost half of the cases, an association with nasal mucosal lesions has 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 mucocutaneous leishmaniasis (MCL) and more closely placed with *L. guyanensis* (Ref. 45), which is also commonly observed in the New World. Classically CL, DSL and MCL are grouped as American tegumentary leishmaniasis in the Americas, especially due to *L. braziliensis* (Ref. 46).

Lupoid leishmaniasis

Lupoid leishmaniasis, also referred to as leishmaniasis rucidivans (LR), is a rare cutaneous form of leishmaniasis that occurs in patients with a strong cellular immune response (Ref. 21). It is caused by the 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 (Ref. 47). It is mostly caused by *L. tropica* in the Old World.

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 (Ref. 48). Clinically, there is early infiltration of the mucosa with superficial ulcerations, and the borders have a necrotic appearance, being 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 parasites 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 (Ref. 41). According to the Centers for Disease Control and Prevention, this condition mainly affects individuals in Bolivia, Brazil, Ethiopia and Peru, with over 90% of cases occurring in these countries (Ref. 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 VL disease form, commonly known as KA or dumdum fever, is a life-threatening disease listed among neglected tropical diseases by the World Health Organization (WHO) (Ref. 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 the organs involved. Clinical manifestation of the disease includes anorexia, lymphadenopathy, hepatomegaly, splenomegaly, pallor, anaemia, 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 (Ref. 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 West Bengal (11 out of 23 districts) (Ref. 51). Sporadic cases are also reported in other states, including Assam, Gujarat, Himachal Pradesh, Jammu and Kashmir, Kerala, Madhya Pradesh, Haryana, Puducherry, Sikkim, Tamil Nadu and Uttaranchal (Ref. 51).

WHO's global leishmaniasis surveillance for 2017–2018, along with additional indicators, underscores the continued importance of monitoring and addressing VL. An estimated 50,000–90,000 new cases emerge annually worldwide (Ref. 40). This stark prevalence emphasises 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 (Ref. 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 KA elimination, the block-level incidence of cases

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needs to be reduced to less than 1 case per 10,000 population. This target aligns with the new NTDs roadmap 2021–2030 (Refs 1, 52). By the end of 2021, 98% of blocks had 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 (Ref. 53). India has hugely expanded vector control interventions. The endemic states need to mandatorily notify cases to the National Vector Borne Disease Control Programme every month, even if there are zero cases (Ref. 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 KA over the past 3 years, two rounds of indoor residual spraying (IRS) are being applied. WHO in coordination with the Ministry of Health and Family Welfare, Government of India and NCVBDC organised a coordinated programme to assess the situation and progress of the KA elimination programme in two endemic states: West Bengal and Uttar Pradesh (Ref. 55). PKDL is a sequel of VL in certain populations following the apparent cure of VL (Ref. 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 the recent challenge of cutaneous cases caused by *L. donovani* variants, are being recognised as an 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 KA. It is a form of CL that usually occurs months to years after VL treatment (Ref. 14). It typically manifests 6 months to a year or more after KA 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 sequelae 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' (Ref. 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 1 year after a bout of VL, adding complexity to the understanding of the disease progression (Ref. 14).

Leprosy and PKDL resemble each other closely in their clinical manifestations. A rapid accurate assay called 'm-LAMP' could be used for the differential diagnosis of leprosy versus PKDL (Ref. 57). In a comparison of treatment susceptibilities between VL and PKDL isolates, the latter displayed reduced susceptibility to miltefosine than the VL isolates (Ref. 58). Towards that end, a combination therapy with liposomal amphotericin B and miltefosine displayed larger efficacy in healing of PKDL (Ref. 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 (Refs 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 (Ref. 61). Active prevalence of Para-KDL has been reported in East Africa, although rare cases have also been documented in India and Brazil (Refs 62, 63). A study identified nine cases in India, mainly from Bihar, linked to relapse from miltefosine treatment (Ref. 62). The challenges of diagnosing Para-KDL contribute to poor prognoses for affected individuals, particularly as parasites show reduced susceptibility to current treatments (Ref. 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 (Ref. 60). Interestingly, three cases of human immunodeficiency virus (HIV)–Para-KDL comorbidities were also observed in Brazil (Ref. 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 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 (Refs 65, 66, 67, 68, 69). Similar cases had been reported from the Himachal regions of India and other northern neighbouring countries (Refs 31, 70, 71, 72). Nevertheless, *L. donovani* is not the sole causative agent of CL in Sri Lanka, potentially explaining a haplotype that resulted in interspecies dermotropic hybrids of *L. donovani* with *L. tropica* (Ref. 69). 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 (Ref. 73). Whole-genome sequence analysis has also shed considerable light on genetic variations and polymorphisms that exist between causative parasites in different regions (Ref. 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 (Ref. 74). In Himachal Pradesh, as a new endemic site for CL caused by *L. donovani*, parasite isolates from CL patients comprise considerable heterogeneity at the genetic level, with accumulation of wide genetic mutations in terms of ploidy changes, copy number variations, InDels and single nucleotide polymorphisms (SNPs) that are different from those detected for *L. donovani* CL isolates from Sri Lanka (Ref. 70).

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* (Refs 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 (Ref. 77) and Bangladesh supported by subsequent molecular confirmation (Ref. 31). Similarly *L. tropica* causing VL has also

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been observed in U.S. soldiers of Operation Desert Storm (Ref. 78). In addition, *L. infantum*, and not *L. donovani* infection, had been reported to have caused PKDL in an HIV-1-infected patient in Australia (Refs 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 (Ref. 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 (Ref. 82). Furthermore, three beagle dogs displaying atypical VL due to *L. infantum* in Europe had been reported with rare granulomatous peritonitis (Ref. 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 such as LACK, A2, Q-protein, GP63, KMP-11 and TYRP (Refs 84, 85, 86, 87, 88, 89). Challenged with such antigens provides 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 used in Brazil and European countries to treat dogs, although they do not work for humans (Refs 90, 91, 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 and Infectious Diseases Society of America suggest close monitoring of asymptomatic individuals with the initiation of treatment only upon symptom development (Ref. 93). Interestingly, these patients have elevated $CD4^+$ T cell counts and test positive for leishmanin skin test (Refs 94, 95). In addition, a 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. (Ref. 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 the 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 the twentieth century has faced stiff resistance over the past decade or two, particularly in areas such as Bihar, India (Refs 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 (Ref. 99). The derivative of antimony, SSG also has been discouraging due to the development of resistance to SSG by the parasite (Ref. 100). The genetically diverse Sb-resistant parasites displayed elevated thiol-synthesising and antimony transporter gene expression compared to the susceptible ones (Ref. 101). As a lesson, antimonials are used in combination with paromomycin as a first-line treatment for VL in East Africa to minimise the chance of resistance development (Refs 102, 103).

Alternatively, lipid-formulated amphotericin B deoxycholate is also being used against VL in the ISC (Indian subcontinent), instead of just amphotericin B deoxycholate, to reduce side effects. However, its high cost has become a major concern, together with a considerable number of relapses noticed in the ISC (Ref. 103). The other commonly known drug is miltefosine, an orally administered medication that has been in use since 2002 in the ISC. However, resistance shown against miltefosine in VL patients has raised significant concerns in recent years (Ref. 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 (Ref. 46). Apart from such pharmacokinetics-based reasons, there could also be parasite's own mechanisms leading to resistance. In addition to such disadvantages, its serious adverse side effects, believed to be due to immunopathological consequences, have led to the discontinuation of its use (Ref. 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 (Ref. 1). Zoonotically, *Leishmania*-infected rodents or sand flies serve as reservoirs of infection for humans. In addition, 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. The 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

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reported along the Sutlej River belt. This could be due to the possible upstream migration of vectors along the rivers (Ref. 105).

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 (Refs 106, 107, 108). Their high similarity results in the anomalous outcomes. Additionally, myosinXXI localisation has been used as a biomarker to distinguish *Leptomonas* in *Leishmania* cultures (Ref. 109). To the best of 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 (Ref. 110). Moreover, the 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 (Refs 111, 112), adding another dimension to the research on the causes of VL in the Indian subcontinent. The detection of *Leptomonas* spp. with a monoxenous life cycle and considered non-pathogenic to humans implies 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 (Ref. 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 (TB), 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 HIV is a life-threatening condition. This is because HIV infection and leishmaniasis together promote the replication of both causative pathogens and accelerate the progression of both VL and HIV (Refs 114, 115). The first reported case of VL/HIV co-infection 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 co-infected with VL/HIV have the highest relapse rate and mortality, which poses significant challenges in the prevention and control of VL (Ref. 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 (Ref. 117). HIV-infected people contracting leishmaniasis are at a high risk of developing the full-blown disease, with high relapse and mortality rates (Refs 118, 119). Antiretroviral treatment is known to reduce the development of the disease, delay relapses and increase the survival rates. As of 2021, *Leishmania*–HIV co-infection 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 (Ref. 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 (Ref. 121) analysed 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 (Ref. 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 (Refs 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 (Ref. 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 TB/pulmonary TB is common and a significant concern in regions where both diseases are endemic, such as parts of Africa, Asia and Latin America (Refs 125, 126). Second to VL, MCL can also co-exist with TB in certain parts of Asia (Refs 127, 128).

Malaria, caused by the apicomplexan protozoan parasites *Plasmodium falciparum* or *P. vivax*, co-infecting with *Leishmania*, has been extensively reported worldwide (Ref. 129). For instance, a 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 (Ref. 130). The prevalence of malaria co-infection with VL varies from 7% to 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 the plastic nature of the parasite genome with the accumulation of newer genetic

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variations. Multilocus sequence typing (Refs 131, 132), randomly amplified polymorphic DNA (Refs 133, 134), microsatellite typing (Refs 135, 136) and restriction fragment length polymorphism (Refs 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 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 epidemiological surveillance. Complete/partial WGS information with nucleotide/gene/protein annotation information on several *Leishmania* species/strains is 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 and *Leishmania* turanica strain LEM423.

A recently described minicircle-based DNA footprint assay has simplified the detection and speciation of *Leishmania* clinical isolates (Ref. 74). This method has enabled the study of phylogenetic relationships and variations of many *Leishmania* species that have originated from different parts of the world. Parasites from CL lesions from red kangaroos of Australia (Ref. 139) were found to be grouped into a 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 (Ref. 74). Therefore, it remains a promising approach for phylogenetic analysis, including the measurement of the phylogenetic distances and the 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 the detection of newer parasite variants circulating in different geographical sites and can help in aligning policymakers for evidence-driven strategies for disease diagnosis, treatment and elimination. Targeting multicopy DNA regions such as 18S rRNA, heat shock proteins or mini- or 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 readout 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 (Ref. 140)), in *Leishmania* together with those of a few other Trypanosomatid parasites. The results are presented in Figure 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 (Figure 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*) (Ref. 141) and one non-human parasite *L. enriettii* (Ref. 142) (Figure 2A). The three non-*Leishmania* parasites (*Trypanosoma brucei, Trypanosoma cruzi* and *L. 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* (Refs 143, 144). Their differences in amino acid percent identity are also compared in Figure 2B,C. In addition, interestingly, based on centrin5, *L. tarentolae*, the parasite that infects exclusively lizards (Ref. 145), has been grouped with CL-causing parasites (Figure 2A). Overall, this study gives us a meaningful grouping of a few of *Leishmania* and other genera of Trypanosomatid family that we attempted via cladogram analysis using the MEGAX program. Such genetic variations across *Leishmania* species/strains circulating in known and newer endemic zones need molecular surveillance for the detection and prediction of region-specific parasite variants and associated disease outcomes.

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 (Ref. 148). Preventing the development of drug resistance is a key aspect of the 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 (Ref. 149). However, it is important to note that each pathogen manipulates different aspects of the host immune response (Ref. 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

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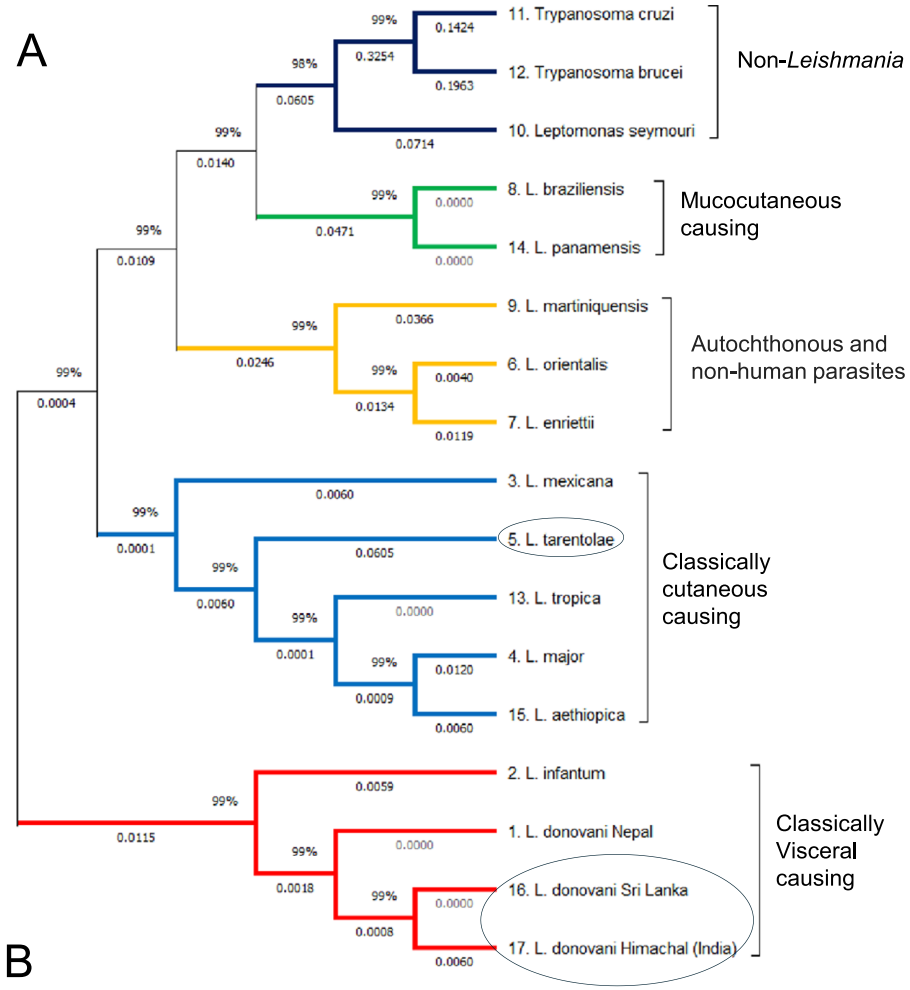
A



B

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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
                               ************************************:******
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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 by distinct colours and labelled on their right. The parasite genera and species are described with sources indicated for some. The accession numbers of centrin5 proteins of the parasites, along with their associated serial numbers, are as follows: 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 and 17. AYU83995. The branch lengths and the bootstrap % values are also shown. The tree was constructed by the Maximum Likelihood method and JTT matrix-based model using the MEGA X program (Refs 146, 147). **B,C.** Multiple-sequence alignment and % identity of centrin5 proteins of only four of the red branches 'A' above using the 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 Material.

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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 have shown promise. These combinations also offer hope for co-infections. In Ethiopia, AmBisome plus miltefosine has proven efficacious in HIV–VL patients. Additionally, improved genetic, immunological and serological markers are needed to determine the progression from parasite infection to clinical VL. Markers for asymptomatic infections have been utilised in clinical studies. However, in the absence of specific safe drugs or markers of disease progression, further research is required to develop newer tools to address these challenges. Various vaccine strategies have also been explored, including those utilising recombinant peptide, DNA, killed whole parasite and genetically modified live-attenuated parasites (Ref. 92). Notably, the *L. donovani* and *L. major* centrin gene knockout strains show promise as a live attenuated vaccine against both VL and CL (Refs 151, 152, 153). Additionally, a clinical trial utilising ChAd63-KH, an adenoviral vaccine encoding KMP-11 and HASPB, was conducted in Sudan with 24 PKDL patients, and the vaccine successfully generated a potent innate and cell-mediated immune response (Ref. 154). The results showed that 30.4% of patients had over 90% clinical improvement, while 21.7% showed partial improvement. Following this, a Phase 2 vaccine trial with ChAd63-KH was conducted on 100 patients with persistent PKDL in Sudan. This vaccine has been proven effective in those patients (Ref. 155).

Periodic genome sequencing of the parasite isolates in affected regions can provide valuable insights into emerging *Leishmania* variants. These data serve as an alert for clinicians and researchers, prompting increased attention towards emerging parasite variants and the associated clinical manifestation in region-specific manner. Implementing effective measures to control vector populations is another crucial approach for achieving the successful elimination of leishmaniasis. Addressing gaps in our understanding of vector bionomics is essential in this regard. These gaps include screening for infected sand flies using PCR, determining sand fly biting rates, assessing parasite infection rates within the vector population and understanding the spatial and temporal variations of these parameters in response to interventions such as IRS. Bridging these knowledge gaps is paramount to achieving sustained elimination of VL and implementing an appropriate post-elimination program. Many countries are now prioritising boosting immunity to prevent infectious diseases, including leishmaniasis, either as a primary infection or as an opportunistic infection alongside other pathogens (Ref. 52). India's significant investment in AYUSH (Ayurveda, Yoga, Unani, Siddha and Homeopathy) as an alternative therapy approach exemplifies this direction. Other immune modulators, such as liposomal cholesterol, which have proven effective experimentally in treating VL, might need further studies. Some countries have transitioned to the post-elimination maintenance phase for leishmaniasis control, emphasising the importance of periodic screenings to detect any reemergence early and prevent resurgence. Although the march towards leishmaniasis elimination appears to be increasing, the achievement of the program remains uncertain in the light of already existing and newly emerging challenges such as sporadic outbreaks, asymptomatic infections and newer changing foci.

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