Chapter

Leishmaniasis

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Abstract

Leishmaniasis is a vector-borne tropical/subtropical disease caused by an intracellular parasite transmitted to humans by sand fly bite. It is endemic in Asia, Africa, the Americas, and the Mediterranean region. Worldwide reports include 1.5–2 million new cases each year, more than 300 million at risk of acquiring the disease, and 70,000 deaths per year. Clinical features depend on the *Leishmania* species and immune response of the host, varying from localized cutaneous disease to visceral form with potentially fatal outcome; however, the common presentation is either cutaneous, mucocutaneous, or visceral leishmaniasis. Many therapeutic agents are being used in *Leishmania* treatment, but the only effective treatment is achieved with current pentavalent antimonials. WHO considers Leishmaniasis as one of the "Neglected Tropical Diseases" that continues to be prevalent despite international, national, and local efforts towards its control and elimination over the last decade. This chapter reviews the global perspective of Leishmaniasis with increasing recognition of emerging "Atypical forms" and new surge of disease across the world mainly due to increasing conflicts in endemic areas leading to forced migration among other causes. All these challenges related to environment, disease, and vector pose major implications on WHO's leishmaniasis control and elimination plan.

Keywords: *Leishmania*, sand fly, vector, parasite, protozoa, phlebotomine

1. Introduction

Leishmaniasis is a vector-borne disease caused by an obligate intracellular protozoa of genus *Leishmania* and is transmitted by the bite of a female phlebotomine sand fly (**Figure 1**). It is a poverty-related disease with an estimated 0.7–1 million new cases reported per year from approximately 100 endemic countries. It is reported from all continents except Australia and Antarctica.

The disease is primarily zoonotic with exception of *L. donovani* and *L. tropica*, although some evidence exists that animal reservoir exists for these species too. There are about 53 species of *Leishmania* described with more than 20 species pathogenic to humans and each distinct species causing different clinical manifestations ranging from self-resolving cutaneous ulcers to disfiguring mucocutaneous lesions to life-threatening systemic visceral disease [1–3]. The outcome depends on multiple factors including parasite characteristics, vector itself, and host factors in a particular patient's immune status. The World Health Organization (WHO) considers leishmaniasis as not only one of the neglected tropical diseases but also a

Figure 1.
This photograph depicts a right lateral view of a Phlebotomus papatasi *sand fly which had landed atop the skin surface of a human volunteer. This specimen had just completed its ingestion of its blood meal, which is visible through its distended transparent abdomen. Sand flies like this* P. papatasi *are responsible for the spread of the vector-borne, parasitic disease, leishmaniasis (courtesy of Centers for Disease Control and Prevention/ prof. Frank Hadley Collins and James Gathany) (https://phil.cdc.gov/Details.aspx?pid=10276).*

public health problem that requires elimination by developing effective therapeutic regimens and prevention/control plans.

2. Epidemiology

The disease classification is complex and can be characterized by either its *clinical presentation* into cutaneous (localized or disseminated), mucocutaneous, and visceral or *geographic location* into *Old World leishmaniasis* mainly including Africa, Asia, the Middle East, the Mediterranean, and India or *New World leishmaniasis* including Central and South America (**Table 1**) [4–6].

More than 90% of the cases of visceral leishmaniasis (VL) cases worldwide were reported from 7 countries in 2015 including Brazil, Ethiopia, Kenya, India, Somalia, Sudan, and South Sudan; however, the disease remains endemic in more than 60 countries [1]. The Indian subcontinent accounts for almost 70% of the world's anthroponotic visceral leishmaniasis cases, India having the highest incidence

Genus	Division	Subgenera	Species	Disease	Geographic Area
Leishmania	Euleishmania	*L. (Sauroleishmania)*	*L. tarentolae*		Old world
			L. adleri		
			L. hoogstraali		
		L. (Leishmania)	*L. enriettii*		
			L. major	Cutaneous leishmaniasis	
			L. gerbilli		
			L. turanica		
			L. arabica		
			L. tropica	Cutaneous leishmaniasis	
			L. aethiopica	Cutaneous leishmaniasis	
			L. donovani	Visceral leishmaniasis	
			L. infantum	Visceral leishmaniasis	
			L. martiniquences		New world
			L. mexicana	Cutaneous leishmaniasis	
			L. amazonensis	Cutaneous leishmaniasis	
			L. aristidesi		
			L. venezualenesis	Cutaneous leishmaniasis	
			L. forattinii		
		L. (Viannia)	*L. braziliensis*	Cutaneous & mucocutaneous leishmaniasis	
			L. peruviana	Cutaneous leishmaniasis	
			L. guyanensis	Cutaneous leishmaniasis	
			L. panamensis	Cutaneous leishmaniasis	
			L. lainsoni		
			L. naiffi		
			L. lindenbergi		
			L. utingensis		
	Paraleishmania		*L. colombiensis*		
			L. equatorensis		
			L. hertigi		
			L. herreri		
			L. deanei		

Table 1.
Leishmania *taxonomy showing most of the clinically significant Leishmania species [1, 5, 6, 14].*

followed by Nepal and Bangladesh. In immunocompromised patients, *Leishmania* parasites can persist for decades after management and may reappear exhibiting fulminant reactivation when immunity is compromised. Between 5 and 50% of

treated VL cases may develop post-kala-azar dermal leishmaniasis (PKDL), depending on geographic location, secondary to the interferon gamma-driven immune response against dermal parasite [7–9].

More than 90% of cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (ML) cases are reported from Pakistan, Afghanistan, Syria, Saudi Arabia, Iran, Brazil, Algeria, and Peru. The number of reported VL cases has decreased substantially in the past decade most likely due to early diagnosis and better access to treatment. In East Africa, however, the fatal disease case number continues to be sustained. However there is a surge in endemic CL across the world predominantly due to increased conflicts with forced displacement of population and living in poor sanitary conditions. In addition, there is also an increase in the number of overall *Leishmania* cases reported worldwide and increased new cases reported from nonendemic areas [10–12]. A renaissance of CL is seen in conflict areas of Middle East in particular Syria mainly due to collapse of public health systems, exposure of nonimmune population, and poor living conditions.

2.1 Risk factors

Population migration of susceptible individuals in endemic areas as well as into nonendemic areas, malnutrition, poverty, and immune status of the host play a major role. Temperature is another important factor as *Leishmania* species for cutaneous disease grow best at lower temperature, while species causing VL grow better at core temperatures.

The pathogenesis appears related to T-cell cytotoxicity. The promastigotes activate complement system through alternate pathway with suppression of cell-mediated immunity against the organism. In self-resolving and asymptomatic patients, T helper type 1 cells (Th1) predominate with interleukin-2 (IL-2), interferon gamma, and IL-12 as important cytokines helping with disease resolution. In visceral and diffuse cutaneous forms, T helper type 2 cells (TH2) predominate with patients exhibiting anergy to the organism. These cells secrete IL-4, IL-5, IL-9, IL-13, and IL-17E/IL-25.

A susceptibility gene in band 22q12 is identified in parts of Sudan with high prevalence of VL [13].

Coinfection of VL with HIV is a major challenge. Both infections share a common immunopathologic mechanism involving macrophages and dendritic cells of reticuloendothelial system, therefore leading to an accelerated progression of both diseases in coinfection.

3. Life cycle

Leishmania is scientifically classified as a “genus” that belongs to the “order” of Trypanosomatidae family, under the “class” of Kinetoplastea, under the “phylum” of Euglenozoa. The parasite *Leishmania* exists in two forms: flagellated promastigote form in sandflies and cultures and nonflagellated amastigote form in animals and humans (**Figure 2**) [14, 15]. The sandflies acquire infection when they bite an animal or human host. The parasite develops over 4–25 days in the sandflies and transforms into a promastigote form where they multiply by binary fission in the midgut and move upwards to the pharynx. Infection is transmitted mainly during days 6–9 after ingestion when there is heavy pharyngeal infection and promastigotes are regurgitated via a bite to the host. The sand fly can regurgitate more than 1000 parasites per bite. In the host some of the flagellates are destroyed, whereas others enter intracellular lysosomal organelles of macrophages of

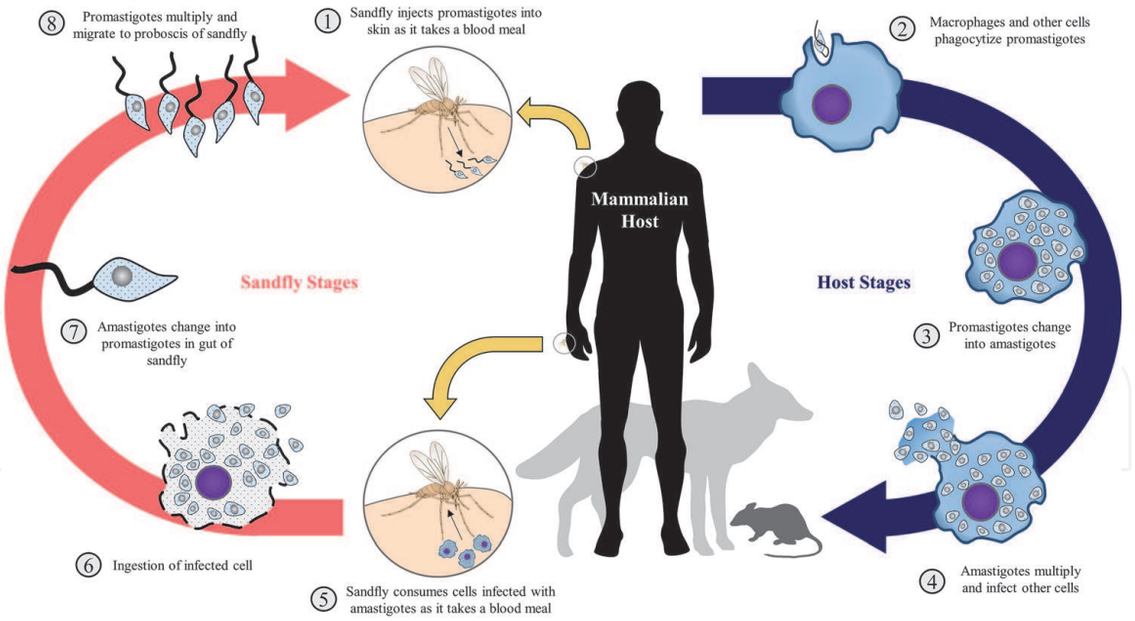

Figure 2.
Leishmania life cycle.

reticuloendothelial system. The flagella of the organisms are lost, and amastigotes are formed that continue to multiply until the infected host cells get filled with organisms and rupture releasing free amastigotes that invade new cells, thus continuing the vicious cycle of *Leishmania* infection (**Figure 2**). The incubation period depends on the individual parasite species. In certain geographic areas, the transmission cycle can be maintained by the infected animals and does not require infected humans. In other areas where disease transmission has the anthroponotic cycle via humans, the disease transmission can be controlled by effective treatment of infected patients [16].

3.1 Mode of transmission

The disease is mainly transmitted by the bite of a 2–3 mm in size female "*Phlebotomus* sand fly" in Old World leishmaniasis and *Lutzomyia* in the New World disease. There are approximately 20 pathogenic *Leishmania* species and up to 500 known phlebotomine sand fly species identified as vectors of disease [17, 18]. The disease is mostly zoonotic with humans being incidental hosts infected by sand fly bite.

4. Clinical presentation

In addition to the known classic disease spectrum and clinical presentations, new unusual and atypical forms are emerging which is adding to the complexity of achieving control and disease eradication goal [19].

4.1 Cutaneous leishmaniasis

CL causing *Leishmania* parasites are divided into New world and Old world species. The New World species affect mainly Central and South America and include *L. amazonensis*, *L. braziliensis*, *L. mexicana,* and *L. guyaensis*, among others. The Old World species examples include *L. tropica, L. major,* and *L. aethiopica* that are common in the Indian subcontinent, the Middle East, the Mediterranean basin, and East

Africa. CL is usually a limited cutaneous disease. The lesions develop as papules at the sand fly bite site and progress over weeks to months to develop larger nodules that eventually ulcerate (**Figure 3**). Lesions are often itchy and may have a hyperkeratotic wart-like appearance. These lesions often self-heal in 2–18 months, leaving a permanent, often disfiguring, scar, leading to major cosmetic concern and social stigma.

Approximately 10% of CL cases may progress to severe disease such as diffuse CL, ML, disseminated CL, and/or *L. recidivans* [1, 20].

4.1.1 Uncommon variants of CL

Uncommonly, CL variants are encountered that are associated with various underlying immune responses.

L. recidivans typically follows a healed *L. tropica* cutaneous infection and presents as new lesions encircling the old scar. The lesions show predominantly

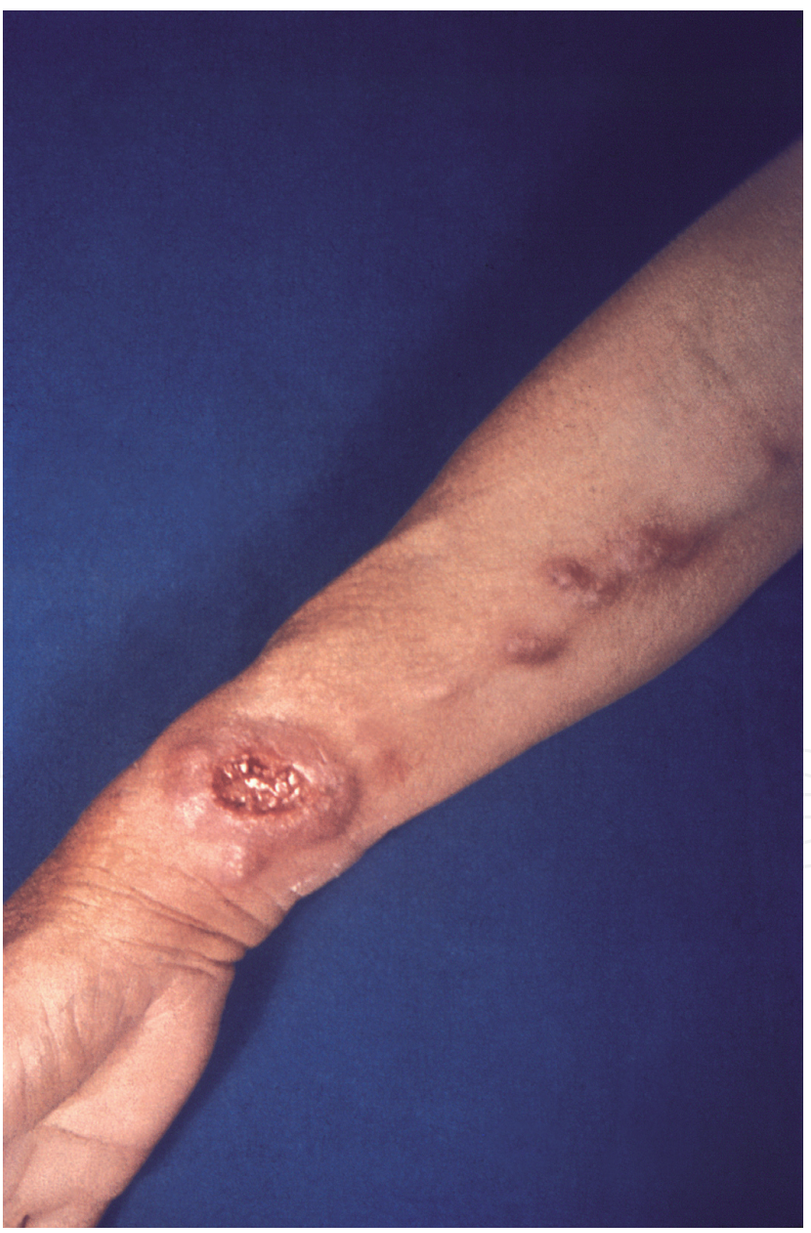

Figure 3.
This photograph depicts the volar surface of a patient's extended right arm, who had been ill with leishmaniasis, having been infected with Leishmania *sp. protozoa, which had manifested itself as a cutaneous form of the disease (courtesy of Centers for Disease Control and Prevention/Dr. martins Castro and Dr. Lucille K. Georg) (https://phil.cdc.gov/Details.aspx?pid=12161).*

increased number of lymphocytes making it difficult to histologically distinguish from tuberculosis.

Diffuse cutaneous leishmaniasis presents with multiple widespread nontender, non-ulcerating lesions, resembling lepromatous leprosy, and a negative leishmanin skin test (LST). The skin is heavily infiltrated by organisms and the patients lack a cellular immune response. These are caused *by L. amazonensis, L. aethiopica, and L. mexicana.*

Disseminated cutaneous leishmaniasis presents with 10 or more mixed-type lesions in multiple body parts and is mostly seen in Latin America with frequent involvement of the mucosa. Histologically, the organisms are scant in the skin lesions, and patients show positive antibodies against *Leishmania* and positive LST test.

Most cases of diffuse cutaneous leishmaniasis and *L. recidivans* are chronic and resistant to treatment, may be exceedingly disfiguring, and can be associated with low mortality rates.

L. infantum/L. chagasi predominantly causes VL; however, it may lead to atypical cutaneous disease. Reported cases are autochthonous, seen in immunocompetent hosts, and diagnosed in different regions [19].

L. donovani is also mainly responsible for VL; however, some atypical autochthonous CL cases by *L. donovani* are reported [19].

4.2 Mucocutaneous leishmaniasis

ML presents as destructive lesions involving oronasal mucosa with involvement of the nasal septum, lips, and palate. Ninety percent of ML cases show a previous CL scar. The disease is often chronic and progressive with destructive, disfiguring midfacial lesions leading to extensive mutilation. Secondary infection and respiratory tract invasion may lead to patient's demise. It is frequently seen in immunocompromised individuals and being a potentially life-threatening disease requires immediate/early diagnosis and treatment [21, 22]. Less than 5% of patients infected by *L. braziliensis* and a small percent of those infected by *L. panamensis* and *L. guyanensis* can develop mucosal involvement months to years after cutaneous disease resolution [13].

4.3 Visceral leishmaniasis

L. donovani is the main species causing VL and humans are the main reservoir for it. *L. infantum* also causes visceral disease; however, it is zoonotic. VL is characterized by a "pentad" of persistent irregular fever, hepatosplenomegaly, weight loss, pancytopenia, and hypergammaglobulinemia. The fever characteristically shows a double rise in 24 hours with spikes of fever and afebrile intervals in between. It is the most devastating and fatal forms of leishmaniasis. The spectrum ranges from asymptomatic infection to fulminant life-threatening disease. The disease may present with an acute or insidious onset, however the typical presentation is that of wasted, thin, cachectic appearance with prominent abdominal distention due to hepatosplenomegaly. Jaundice is considered to be a bad prognostic sign. The incubation period is 2 weeks–8 months. High parasite burden is often associated with malnutrition and wasting in particular in children [23, 24]. VL is often associated with hyperpigmentation of the skin most likely secondary to production of adrenocorticotropic hormones. In such cases it is referred to as kala-azar/black fever [25]. VL, if untreated, is fatal within 2 years; mortality is mostly due to secondary bacterial infection, immunosuppression, hemorrhage due to hematopoietic infiltration, and severe anemia [13].

4.3.1 Uncommon variants of VL

Gulf War soldiers: An uncommon form of VL is described in some US veterans who were infected while participating in Gulf war. These patients had only mild symptoms and light parasitic burden. *L. tropica* was identified as the causative agent in some of these cases [19, 26].

Viscerotropic leishmaniasis: This is an indolent form of disease that has a distinct clinical presentation; however it does not progress to or develop classic VL.

VL-HIV coinfection: HIV is considered to be responsible for the re-emergence of the VL. Both organisms share common pathologic immunologic system involving the reticular endothelial system, therefore leading to accelerated progression of the disease. VL in an HIV-infected person should be considered an acquired immune deficiency syndrome (AIDS)-defining illness, and HIV testing should be mandatory in all patients presenting with VL [1, 27]. Atypical disseminated leishmaniasis can be seen in these patients with lesions involving the gastrointestinal tract and the respiratory tract [21, 28].

L. tropica*, L. amazonensis, and* L. major generally associated with CL are reported to be viscerotropic and uncommonly may lead to visceral disease [19].

4.4 Post-kala-azar dermal leishmaniasis

PKDL was commonly seen in India and Africa as a late complication of VL secondary to *L. donovani* and rarely *L. infantum,* the latter typically in immunocompromised patients [29]. Patients often present months to as many as 20 years after VL with hypopigmented or erythematous skin lesions which over time progress to develop plaques and nodules over the face and trunk. These lesions are often nontender and have preserved sensation, a feature distinguishing this from lepromatous leprosy. The lesions often resolves spontaneously; however, relapse is common, and resistant forms to antimonial treatment have been reported [1, 13].

5. Diagnosis

Centers for Disease Control and Prevention (CDC) has a practical guide for laboratory diagnosis of leishmaniasis at http://www.cdc.gov/parasites/leishmaniasis/health_professionals/index [18].

Cutaneous and mucocutaneous lesions usually show normal values in routine laboratory testing. VL, on the other hand, may exhibit normocytic normochromic anemia, leukopenia, and/or thrombocytopenia due to bone marrow or spleen involvement. In addition there may be involvement of other organs leading to their respective abnormal functions such as abnormal liver function test in patients with significant hepatic disease.

Characteristically the diagnosis is confirmed by visualizing the amastigote form of the protozoa from infected tissue by performing invasive procedure such as dermal scraping or biopsies for cutaneous lesions and/or fine-needle aspirates/biopsies for visceral disease (**Figure 4**). The smears are often stained with Giemsa, Leishman, and/or Wright's stain and slides reviewed under oil immersion. Historically, splenic puncture was considered the most sensitive method and golden standard procedure; however it is potentially life-threatening, carries a high risk of complications such as hemorrhage, and therefore currently is considered unnecessary. In endemic areas with high clinical suspicion, clinical history and physical examination is often sufficient to reach the diagnosis. Since the localized disease has

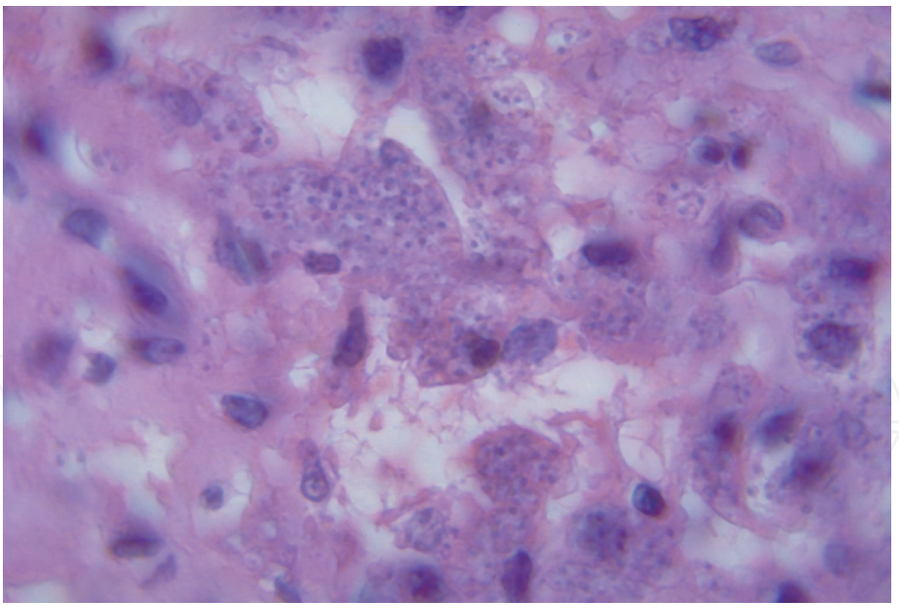

Figure 4.
This photomicrograph of a subcutaneous tissue sample reveals the presence of numerous Leishmania donovani *parasites (courtesy of Centers for Disease Control and Prevention/Dr. Martin D. Hicklin) (https://phil.cdc.gov/Details.aspx?pid=330).*

prominent cell-mediated immunity to the organism, especially when long-standing, isolation, identification, and culture of these organisms can be extremely challenging. In cutaneous disease the organism may be visualized in samples obtained by biopsy, scraping, or FNA in approximately 70% of cases, while the culture from the skin shows only 40% sensitivity [13, 30].

A skin punch biopsy is recommended for the CL to be taken from the raised edge of an active lesion where parasites exist. In addition to the formalin-fixed sections, touch preparations/tissue impression slides can also be prepared and examined. The diagnostic finding is to identify the amastigotes with their eosinophilic rod-like cytoplasmic kinetoplast (**Figures 5** and **6**). In long-standing lesions, biopsy of the necrotic center of the lesion, and cases with low burden disease, the biopsy may show false-negative results.

Mucosal biopsies and/or dental scrapings are used for mucocutaneous lesions to look for organisms.

Invasive procedures such as aspirates or biopsies from the spleen, bone marrow, lymph node, and/or liver were used to diagnose VL. However, technology advancement and development of rapid diagnostic tests (RDT) such as recombinant K39 assay with its high sensitivity and specificity made the above invasive procedures unnecessary. In general, the positivity rate for identification of amastigotes in splenic aspirate is 98% and in bone marrow aspirate/biopsy is 54–86% [31, 32]. Blood samples, except in HIV-infected patients, and lymph nodes have lower sensitivity.

Leishmanin skin test/Montenegro skin test, similar to purified protein derivative (PPD) test used for *Mycobacterium tuberculosis*, is a marker of cellular immune response and tests for delayed-type hypersensitivity reaction. The test uses injection of killed promastigotes in the skin. If there is a skin induration of at least 5 mm after 48–72 hours, the test is considered positive. The test is negative in acute infection as it shows positive results after 2–3 months of infection. In addition the test is negative in active VL and immunosuppressed patients due to anergic response. In the United States, no skin tests for leishmaniasis are approved because of lack of standardization; however, it is used in developing countries and is useful in epidemiological surveys as a marker of previous exposure [1].

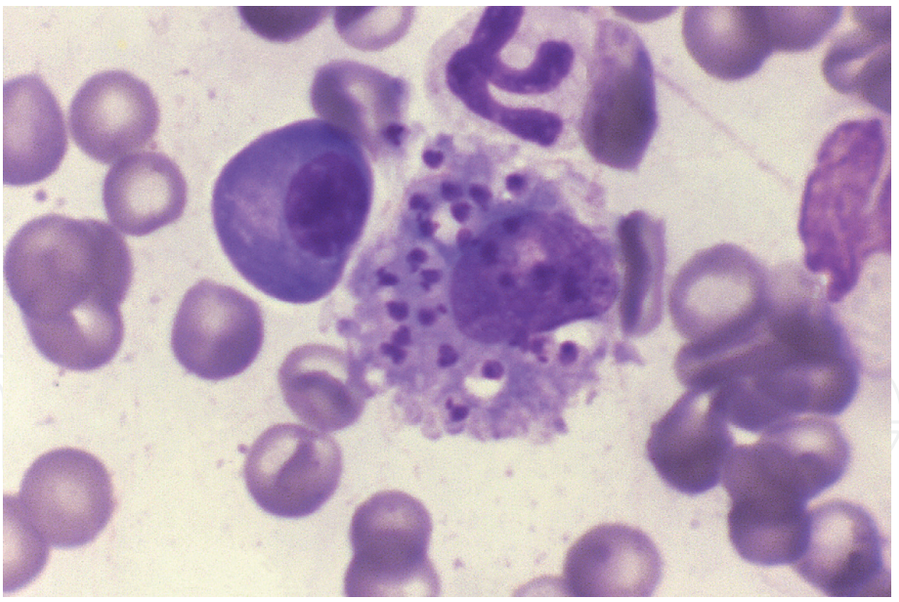

Figure 5.
This photomicrograph depicts some of the histopathologic details seen in a canine bone marrow smear, processed using Giemsa stain, in the case of leishmaniasis. This particular view displays Leishmania donovani *parasites contained within one of the bone marrow histiocytes (courtesy of Centers for Disease Control and Prevention/Dr. Francis W. Chandler) (https://phil.cdc.gov/Details.aspx?pid=30).*

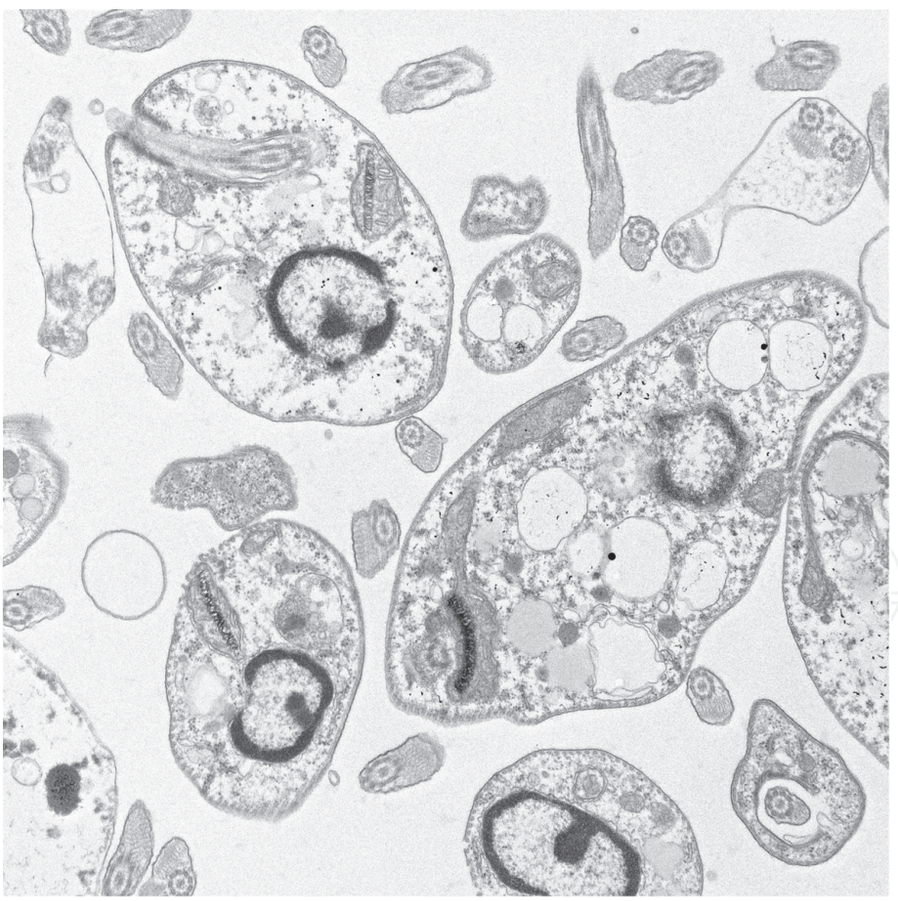

Figure 6.
This is a transmission electron microscopic image of Leishmania major *amastigotes, which had been grown in a cell culture. Note the dense kinetoplasts in the cytoplasm (courtesy of Centers for Disease Control and Prevention/Cynthia goldsmith and Luciana Flannery) (https://phil.cdc.gov/Details.aspx?pid=22001).*

Several serological assays for detection of antibodies against leishmaniasis have been developed using various techniques such as direct agglutination (DAT), immunofluorescence assay (IFA), enzyme-linked immunosorbent

assay (ELISA), and western blot. Although these tests show high sensitivity for acute VL, they are not specific for this disease and may show false positivity with other organisms.

Detection of antibodies to recombinant K 39 antigen appears to correlate with active VL disease in species such as *L. donovani, L. chagasi*, and *L. infantum*. These RDTs however are not useful in cutaneous and mucocutaneous infection. Based on a Cochrane review of RDTs, the sensitivity for rK39 RDT assay is excellent at 97% in Indian subcontinent but low in East Africa and Sudan at 85%. More recently, an rK28 antigen-based RDT shows better sensitivity in Sudan [33, 34]. Recent efforts in developing tests that detect antigens show promising results but still with certain limitations; latex agglutination test has moderate sensitivity of 64% and higher specificity of 93%, while most recent ELISA test shows more than 90% sensitivity [1, 35].

Molecular techniques including polymerase chain reaction (PCR), with significant advances in technology, show higher sensitivity; however, due to the higher cost and complexity of the procedure, they are not available in resource-limited settings. This is particularly true in VL. These tests have a higher sensitivity for cutaneous lesions: reverse transcriptase loop-mediated isothermal amplification (LAMP) technology exhibiting a sensitivity of 98% in CL.

6. Treatment

Multiple factors play a role in treatment decision-making for *Leishmania* that include the specific species, geographic location, comorbidities, and the type of disease whether CL, ML, PKLD, or VL. On most part, it is considered as a treatable and curable disease; however it requires an immunocompetent system. Despite multiple efforts and after all these years, the treatment of *Leishmania* still remains a problem. This is mostly because of indiscriminate treatment leading to frequent emergence of parasite resistance, and the side effects of antileishmanial therapeutic agents call for a search for alternative treatment including the use of natural products such as plants and herbs [36]. Traditionally, medicinal plants have been used throughout the history and are still being used as an alternative therapy to conventional health care, in particular in developing countries and mainly in rural areas that are often deprived of public health resources [37]. This science of using medicinal plants as therapeutic agents is referred to as phytotherapy [38]. The alternate therapies, when studied, show different mechanisms of action. Plants have several secondary metabolites, for example, flavonoids, polysaccharides, lactones, alkaloids, diterpenoids, and glycosides that may activate the immunological system [39]. As an example, a combination of miltefosine and nanoparticles of curcumin, a component of turmeric, displayed lymphocyte proliferation and increased the phagocytic capacity of peritoneal macrophages [40]. Another example is tricin which is isolated from *Casearia arborea*, an evergreen tea, that was reported to modulate the respiratory burst, thus helping in parasite elimination.

Other mechanisms reported as possible mechanisms of action include reactive oxygen species generation and apoptosis-inducing potential. Examples of the latter include ethanolic extract of seeds and leaves of *Azadirachta indica* and essentials oils of *Artemisia campestris* and *Artemisia herba-alba* that act as an apoptosis inductor in promastigotes of *L. donovani* and *L. infantum* [36].

Several studies have been carried out to assess the efficacy of such alternate treatment; however, the results have not been very encouraging. Most of the plants show immunomodulatory effect, but no leishmanicidal effect has been validated,

supporting the notion that substances obtained from plants may complement the treatment of leishmaniasis because of their immunomodulatory effects, but there is no direct effect against the parasite.

There are recommended guidelines for *Leishmania* treatment by the WHO [16]. In addition, a panel of the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH) have developed management guidelines for *Leishmania* patients. These guidelines are mainly for physicians practicing in North America and are based, whenever possible, on randomized clinical trials and a systematic method of grading the quality of evidence and strength of recommendation [41].

6.1 Cutaneous leishmaniasis

Most CL cases spontaneously regress in immunocompetent hosts over 2–18 months, and therefore conservative approach can be used in particular for those caused by *L. major* and *L. mexicana*. On the other hand, *L. braziliensis* has a low spontaneous cure rate. The decision to treat CL and ML is often to reach a goal of reducing the risk of disfigurement, scarring, dissemination, accelerating cure, and subsequent progression to mucocutaneous disease in cases of CL. In addition it is important to classify cutaneous lesions into simple or complex cutaneous lesions based on certain criteria that are then used for treatment decision-making process. These include immunocompetent versus immunocompromised host status, regional lymphadenopathy, multiplicity of lesions (>4), lesions of >5 cm in size, lesions on sensitive areas (such as face, ears, eyelids, lips, fingers/toes, genitalia, or joints), more than 6 months duration, and *Leishmania* species that are more likely to be associated with ML, unusual presentations such as diffuse or disseminated CL, and *L. recidivans*. Traditional treatment for CL has been intralesional injections mostly sodium stibogluconate, thermotherapy, cryotherapy, and topical agents such as paromomycin. The combination of intralesional antimonials and cryotherapy is often the first-line treatment option for CL, resulting in higher cure rates. There has been lack of standardization and poor trial designs for therapeutic regimens in the past. Recently efforts are being carried out to develop unified criteria to define measurable endpoints for different treatment regimens. The treatment regimen decision regarding whether to give local or systemic therapy or choice of therapeutic modality is based on the geographic location and the infecting *Leishmania* species.

Local treatment includes a combination of intralesional antimonials and cryotherapy, paromomycin ointment containing methylbenzethonium chloride, paromomycin containing 0.5% gentamicin, and paromomycin with allopurinol for *L. recidivans*.

Systemic regimens include oral fluconazole, pentavalent antimonials with or without pentoxifylline, ketoconazole, miltefosine, liposomal amphotericin B (LAMB), and pentamidine isethionate.

Systemic treatment for CL is usually used for immunosuppressed patients, mucocutaneous lesions, diffuse/extensive lesions, and refractory disease. In addition infection by *L. braziliensis* and *L. infantum* should be considered for systemic treatment (**Tables 2** and **3**) [1, 17, 18].

6.2 Mucocutaneous leishmaniasis

For mucocutaneous disease systemic regimens include pentamidine isethionate, pentavalent antimonials + pentoxyfilline, and LAMB (**Table 4**) [17, 18].

Leishmania species	Local therapy	Systemic therapy	Relapse treatment
L. Mexicana	**1.** 15% paromomycin and 12% methylbenzethonium chloride ointment twice daily for 20 days (B) **2.** Thermotherapy: 1–3 sessions with localized heat (50 °C for 30 s) (A) **3.** Intralesional antimonials: 1–5 ml per session every 3–7 days (1–5 infiltrations) (B)	**1.** Ketoconazole: adult dose, 600 mg oral daily for 28 days (B) **2.** Miltefosine: 2.5 mg/kg per day orally for 28 days (B)	**1.** Amphotericin B deoxycholate, as above **2.** Pentavalent antimonials: as above plus topical imiquimod every other day for 20 days (A) **3.** Liposomal amphotericin B: 3 mg/kg per day, by infusion, up to 20–40 mg/kg total dose may be considered
L. guyanensis and L. panamensis	(same treatment regimen as *L. mexicana*)	**1.** Pentamidine isethionate, intramuscular injections or brief infusions of 4 mg salt/kg per dose every other day for 3 doses (C) **2.** Pentavalent antimonials: 20 mg Sb5+/kg per day intramuscularly or intravenously for 20 days (C) **3.** Miltefosine: 2.5 mg/kg per day orally for 28 days (B)	(same treatment regimen as *L. mexicana*)
L. braziliensis	(same treatment regimen as *L. mexicana*)	**1.** Pentavalent antimonials: 20 mg Sb5+/kg per day intramuscularly or intravenously for 20 days (A) **2.** Amphotericin B deoxycholate: 0.7 mg/kg per day, by infusion, for 25–30 doses (C) **3.** Liposomal amphotericin B: 2–3 mg/kg per day, by infusion, up to 20–40 mg/kg total dose (C)	(same treatment regimen as *L. mexicana*)
L. amazonensis, L. peruviana, and L. venezuelensis	(same treatment regimen as *L. mexicana*)	**1.** Pentavalent antimonials: 20 mg Sb5+/kg per day intramuscularly or intravenously for 20 days	(same treatment regimen as *L. mexicana*)

Table 2.
Treatment regimens for cutaneous leishmaniasis, New World species, as per WHO recommendations (adopted from WHO) (https://www.who.int/leishmaniasis/research/978924129496_pp67_71.pdf?ua=1) [8].

Leishmania species	Local therapy	Systemic therapy
L. major	**1.** 15% paromomycin/12% methylbenzethonium chloride ointment twice daily for 20 days (A) **2.** Intralesional antimonials, 1–5 ml per session plus cryotherapy (liquid nitrogen: – 195 °C), both every 3–7 days (1–5 sessions) (A) **3.** Thermotherapy, 1–2 sessions with localized heat (50 °C for 30 s) (A) **4.** Intralesional antimonials or cryotherapy independently, as above (D)	**1.** Fluconazole, 200 mg oral daily for 6 weeks (A) **2.** Pentavalent antimonials, 20 mg Sb5+/kg per day intramuscularly or intravenously for 10–20 days (D) **3.** Pentavalent antimonials, 20 mg Sb5+/kg per day intramuscularly or intravenously plus pentoxyfylline, 400 mg three times a day for 10–20 days (A)
L. tropica and L. infantum	**1.** 15% paromomycin/12% methylbenzethonium chloride ointment, as above (D) **2.** Intralesional antimonials plus cryotherapy, as above (D) **3.** Thermotherapy, as above (A) **4.** intralesional antimonials, alone, as above (B) **5.** Cryotherapy, alone, as above (C)	**1.** Pentavalent antimonials, 20 mg Sb5+/kg per day intramuscularly or intravenously for 10–20 days (D) **2.** Pentavalent antimonials, 15–20 mg Sb5+/kg per day intramuscularly or intravenously for 15 days plus oral allopurinol 20 mg/kg for 30 days, to treat leishmaniasis recidivans caused by L. tropica (C)
L. aethiopica	(same treatment regimen as *L. tropica* and *L. infantum*)	**1.** Pentavalent antimonials 20 mg Sb5+/kg per day intramuscularly or intravenously plus paromomycin, 15 mg (11 mg base)/ kg per day intramuscularly for 60 days or longer to treat diffuse cutaneous leishmaniasis (C)

Table 3.
Treatment regimens for cutaneous leishmaniasis, Old World species, as per WHO recommendations (adopted from WHO) (https://www.who.int/leishmaniasis/research/978924129496_pp67_71.pdf?ua=1) [8].

6.3 Post-kala-azar dermal leishmaniasis

Treatment regimens for PKDL are scant. In general, majority of cases from East Africa are self-healing and therefore do not require treatment. In contrast, in the Indian subcontinent, these patients are treated. Since vast majority of these patients are healthy and the risk is cosmetic, the risk benefit should be weighed before initiating therapy. Selected treatment regimens are recommended by the WHO,

Recommended treatment regimens for all cases of mucocutaneous leishmaniasis
1. Pentavalent antimonials: 20 mg/kg per day intramuscularly or intravenously for 30 days (C)
2. Pentavalent antimonials: as above plus oral pentoxifylline at 400 mg/8 h for 30 days (A)
3. Amphotericin B deoxycholate: 0.7–1 mg/kg by infusion every other day up to 25–45 doses (C)
4. Liposomal amphotericin B: 2–3 mg/kg daily by infusion up to a total dose of 40–60 mg/kg (C)
5. In Bolivia: miltefosine at 2.5–3.3 mg/kg per day orally for 28 days (B)

Table 4.
Treatment regimens for mucocutaneous leishmaniasis as per WHO recommendations (adopted from WHO) (https://www.who.int/leishmaniasis/research/978924129496_pp67_71.pdf?ua=1) [8].

when patients require treatment, that include miltefosine, amphotericin B deoxycholate, and LAMB mainly for Indian subcontinent. As for East Africa, the WHO based on evidence grading recommends pentavalent antimonial, LAMB, miltefosine, and combination treatment (pentavalent antimonial with paromomycin) (**Table 5**) [17, 18].

6.4 Visceral leishmaniasis

Traditionally VL has been treated by pentavalent antimonials. Recently there is emergence of resistance in the Indian subcontinent. Current recommendations for VL in East Africa include pentavalent antimonials, LAMB, or combination treatment (including pentavalent antimonials with paromomycin). As for the Indian subcontinent, the recommendations include LAMB, amphotericin B deoxycholate, miltefosine, and one of the combination therapies: LAMB with miltefosine, LAMB with paromomycin, or miltefosine with paromomycin [1]. As for complicated VL, elderly patients, and pregnant patients in East Africa, it is recommended to have LAMB treatment because of its better safety. LAMB monotherapy is not recommended in patients with less severe disease in Asia due to lack of proven efficacy in that region [42]. In Asia, sodium stibogluconate, rather than LAMB, is considered the first-line treatment for *L. infantum* and *L. donovani*. The WHO recommended LAMB therapy in the initial elimination phase for *L. donovani* in

Geographic areas affected by post-kala-azar dermal leishmaniasis	Recommended treatment regimens ranked by preference
East Africa	1. Pentavalent antimonials: 20 mg Sb5+/kg per day intramuscularly or intravenously for 30–60 days, when indicated (C) 2. Liposomal amphotericin B: 2.5 mg/kg per day by infusion for 20 days, when indicated (C)
Bangladesh, India, and Nepal	1. Amphotericin B deoxycholate: 1 mg/kg per day by infusion, up to 60–80 doses over 4 months (C) 2. Miltefosine orally for 12 weeks at dosage as above in visceral leishmaniasis (A)

Table 5.
Treatment regimens for post-kala-azar dermal leishmaniasis as per WHO recommendations (adopted from WHO) (https://www.who.int/leishmaniasis/research/978924129496_pp67_71.pdf?ua=1) [8].

Geographic areas affected by visceral leishmaniasis	Leishmania species	Recommended treatment regimens ranked by preference
Bangladesh, Bhutan, India and Nepal	*L. donovani*	**1.** Liposomal amphotericin B: 3–5 mg/kg per daily dose by infusion given over 3–5 days period up to a total dose of 15 mg/kg (A) by infusion or 10 mg/kg as a single dose by infusion (A) **2.** Combinations (co-administered) (A): • Liposomal amphotericin B (5 mg/kg by infusion, single dose) plus miltefosine (daily for 7 days, as below) • Liposomal amphotericin B (5 mg/kg by infusion, single dose) plus paromomycin (daily for 10 days, as below) • Miltefosine plus paromomycin, both daily for 10 days, as below **3.** Amphotericin B deoxycholate: 0.75–1.0 mg/kg per day by infusion, daily or on alternate days for 15–20 doses (A) **4.** Miltefosine: for children aged 2–11 years, 2.5 mg/kg per day; for people aged ≥ 12 years and < 25 kg body weight, 50 mg/day; 25–50 kg body weight, 100 mg/day; > 50 kg body weight, 150 mg/day; orally for 28 days (A) **OR** Paromomycin: 15 mg (11 mg base) per kg body weight per day intramuscularly for 21 days (A) **5.** Pentavalent antimonials: 20 mg Sb5+/kg per day intramuscularly or intravenously for 30 days in areas where they remain effective: Bangladesh, Nepal and the Indian states of Jharkhand, West Bengal and Uttar Pradesh (A)
East Africa (Ethiopia, Eritrea, Kenya, Somalia, Sudan and Uganda) and Yemen	*L. donovani*	**1.** Combination: pentavalent antimonials (20 mg Sb5+/kg per day intramuscularly or intravenously) plus paromomycin (15 mg [11 mg base] per kg body weight per day intramuscularly) for 17 days (A) **2.** Pentavalent antimonials: 20 mg Sb5+/kg per day intramuscularly or intravenously for 30 days (A) **3.** Liposomal amphotericin B: 3–5 mg/kg per daily dose by infusion given over 6–10 days up to a total dose of 30 mg/kg (B) **4.** Amphotericin B deoxycholate: 0.75–1 mg/kg per day by infusion, daily or on alternate days, for 15–20 doses (A) **5.** Miltefosine orally for 28 days at dosage as above (A)
Mediterranean Basin, Middle East, Central Asia, South America	*L. infantum*	**1.** Liposomal amphotericin B: 3–5 mg/kg per daily dose by infusion given over a 3–6 days period, up to a total dose of 18–21 mg/kg (B) **2.** Pentavalent antimonials: 20 mg Sb5+/kg per day intramuscularly or intravenously for 28 days (B) **3.** Amphotericin B deoxycholate: 0.75–1.0 mg/kg per day by infusion, daily or on alternate days for 20–30 doses, for a total dose of 2–3 g (C)

Table 6.
Treatment regimens for visceral leishmaniasis as per WHO recommendations (adopted from WHO) (https://www.who.int/leishmaniasis/research/978924129496_pp67_71.pdf?ua=1) [8].

the Indian subcontinent; however currently several combination regimens are available which will alleviate the risk of resistance development to LAMB therapy (**Table 6**) [17, 18].

6.5 Human immunodeficiency virus (HIV): Visceral leishmaniasis coinfection

VL should be treated as an opportunistic infection if diagnosed in patients with HIV, warranting lifelong antiretroviral therapy regardless of CD4 count [27]. The core infected patients require longer treatment with higher doses since they are at a higher risk for disease relapse, poor outcome, and increased mortality. In addition, developing VL disease in HIV patients adversely affects their response to antiretroviral therapy [43]. Current WHO recommendation, so far, is LAMB for all regions. Some cases may require combination treatment including LAMB with mitefosine, pentavalent antimonials, and/or amphotericin B deoxycholate. Randomized control trials are ongoing in both Ethiopia and India comparing combination of LAMB and miltefosine with LAMB monotherapy [44, 45].

6.6 Surgical intervention

Surgical intervention is not the recommended modality of treatment in majority of leishmaniasis cases; however, surgery may be required in certain cases such as splenectomy in resistant disease, orofacial surgery for severely debilitating ML, and cosmetic surgery for disfiguring cutaneous lesions.

7. Prevention and control

L. donovani is perhaps one of the most virulent *Leishmania* species and is present in South Asian region, one of the highest incidence areas of VL. Between 2005 and 2013, *Leishmania* ranked the second worst next only to malaria among the 16 categories of "neglected tropical diseases" [46]. In 2005 the WHO and the government representatives of India, Nepal, and Bangladesh signed a memorandum of understanding with commitment to mutually cooperate in order to achieve VL elimination from these countries by 2015. The objective was to reduce the annual incidence of VL to below 1/10,000 inhabitants by 2015 using detection and treatment of VL cases and vector control measures [47]. The target was not achieved by the expected date of 2015 due to high cost and limited availability of treatment, lack of effectiveness of vector control measures, emergence of parasite resistance, and low community coverage of health services in all areas. A second target was set for 2017; however, the WHO has recently reset the target of VL elimination from the Indian subcontinent to year 2020 [48]. In the Eastern Mediterranean region, a specific target was set for CL to detect 70% of all cases and at least treat 90% of them. There is crucial gap in planning elimination of VL in Asia and VL and CL in other regions. These elimination campaigns will require more intense work and better strategic planning in addition to the high cost required. Currently, there is increased global awareness about the disease and the dire need for its elimination, in particular, after Asian elimination initiative of VL and 2012 London declaration on neglected tropical diseases. Having said that, many challenges still exist that counteract these efforts. These include poor sanitary conditions, conflict zones leading to forced migration, emerging atypical variants, poor public awareness especially in nonendemic areas, suboptimal diagnostic modalities, and limited treatment options [1, 49].

7.1 Mass treatment

The VL elimination initiative in the Indian subcontinent in collaboration with the WHO was based on diagnosis and treatment of VL patients using mass treatment to reach the target of reducing the annual VL incidence to below 1/10,000. This plan was dependent on actively looking for and diagnosing *Leishmania* patients. However, the target date was missed because of several factors including limitations of diagnostic tools to diagnose patients actively, lack of health-care coverage in certain areas in developing countries in particular rural regions, lack of proper vector control, and high cost and limited availability of treatment [46–48].

7.2 Vector control strategies

Including insecticide-treated nets and indoor insecticide sprays are used for areas where sandflies bite indoor. Recently resistance to dichlorodiphenyltrichloroethane (DDT) is reported to emerge, and therefore other synthetic products such as pyrethroids started to be used [50]. In areas like Africa where the vector mainly bites outdoor, selective outdoor spraying might be effective in reducing vector density. In addition alternative vector control measures have been proposed and used such as plastering of walls and floors using mud and lime. However these environmental management methods need further evaluation and validation. The KALANET project was the only trial that evaluated the impact of "long-lasting insecticidal nets" on *L. donovani* and concluded that these nets have beneficial effects against *L. donovani* as they provide some degree of personal protection against infection as compared to those using untreated nets or no nets. Further prospective studies are needed to evaluate integrated vector management measures on VL and other vector-borne diseases [47].

7.3 Reservoir eradication/control

In areas of zoonotic transmission should be effectively targeted to reduce the human infection rate from infected animal reservoir. Several reservoir control measures have been used including animal elimination in certain areas, canine vaccines, and insecticides used on dogs such as spot-on insecticide which are drops applied on skin under the hair in the neck region, insecticide-impregnated dog collars, and whole body insecticide use. Studies on efficacy of animal reservoir intervention programs are limited and show lack of generalizability of intervention measures as well as mixed results [17]. In addition there are also conflicting results on the impact of dogs in transmission of leishmaniasis since not all infected dogs become infectious. All the above factors point towards a fundamental gap in our knowledge of disease biology and its transmission.

7.4 Minimizing outdoor exposure

At dawn to dusk which are the peak bite times and use of insecticide-treated nets and/or fine-mesh nets since the sandflies are small in size and can pass through standard mosquito nets.

7.5 Transmission via blood

Infected patients should not donate blood or organs since the parasite can be transmitted through blood.

7.6 Immunization/vaccination

Several candidate vaccines are in preclinical development, and at least three are currently in clinical studies; however, no effective vaccine has been identified to date to effectively prevent human leishmaniasis [51]. Some studies show vaccination by killed *Leishmania* promastigotes, and live BCG can develop protection against CL, but no protection is seen against VL. Approximately 90–98% of leishmaniasis patients recover after disease and develop natural acquired immunity mainly due to Th1 lymphocyte activation and its reaction towards the infecting parasite. This strongly supports the ongoing vaccination development efforts, hopefully looking forward for a clinically efficient vaccine to be available in the near future.

All the above measures have shown some success; however, they are costly and require extensive coordination efforts globally. Early diagnosis and treatment remain the main control strategy since untreated patients serve as reservoirs of parasites. In most countries majority of patients present themselves to the health care, suggesting that many cases will remain in the community for long periods before seeking health care due to reduced awareness. Strategy for eradication would require surveillance with early detection and prompt treatment measures applied globally, mostly in heavily infested areas.

7.7 Postinfection immunity

Successfully treated patients who receive full course of therapy by effective agents and self-resolving infections generally acquire immunity from the infecting species in 97–98% of the cases.

7.8 Long-term monitoring

Prolonged monitoring and follow-up evaluations of patients after successful treatment are recommended for relapse or recurrence of the disease. Yearly follow-up is recommended for patients infected with *L. braziliensis* for up to a decade for early detection of any progression to mucocutaneous disease. Certain complex cases of ML, diffuse CL, *L. recidivans*, and PKDL can be difficult to treat and may require prolonged therapy. In addition, retreatment and/or second-line medications may be required for patients with resistant disease.

8. Conclusion

There has been increasing global awareness about leishmaniasis and the need for its eradication. However, there are many challenges that hinder this global initiative and maintain leishmaniasis as one of the neglected tropical diseases. These challenges include but are not limited to high cost, variability of clinical spectrum, cyclic transmission patterns, changing disease foci, emerging atypical and resistant forms, suboptimal diagnostics, limited treatment options/availability, and suboptimal community awareness and health-care coverage, in particular in nonendemic areas. Several preventive measures using various strategies are needed to tackle personal human protection against infection, interventions targeting vector and animal reservoir control. With the current known challenges and limitations of resources, perhaps integrated approach to control this infection and focus on development of effective vaccine for protection may be a strategic way to use the limited resources available to reach the WHO's set target of leishmaniasis reduction/elimination [17].

For WHO to reach its leishmaniasis elimination target, seriously committed global efforts with substantial funding will be required. However, the question whether complete accomplishment of this goal is technically achievable, given the abovementioned challenges, remains to be answered.

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