

## Review

# Leishmaniasis: A neglected tropical disease

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Leishmaniasis refers to a diverse spectrum of clinical syndromes caused by infection with protozoan parasites of the genus *Leishmania*. They are widely distributed across the tropical, subtropical, and temperate regions in 88 countries, 72 of which are in developing areas of the world. The diagnosis and treatment of the different syndromes produced by these parasites are particularly difficult in developed and non-endemic countries because of poor

knowledge of clinical symptoms, diagnostic possibilities, and available treatment options. This article highlights the biology, life cycle, diagnostic, chemotherapeutic, and strategies to control Leishmaniasis.

**Keywords:** *Leishmaniasis*; biology; life cycle; diagnosis; treatment; prevention

## INTRODUCTION

Leishmaniasis is a neglected vector-borne tropical infection that is considered to be a disease of the poor (WHO, 2014). It is widely distributed across the tropical, subtropical, and temperate regions; extending from China across Asia, India, the Arabian Peninsula, the Middle East, the Mediterranean basin, East and West Africa and South America (Pace, 2014). Three hundred fifty million women, men, and children are at risk in widely scattered areas, with 14 million people directly affected by the disease (Pace, 2014). Based on the pattern of organ pathology, leishmaniasis is sub-divided into three clinical types, namely visceral (kala-azar), the most important disease; cutaneous, the most common; and mucocutaneous. Among parasitic diseases, mortality from leishmaniasis is second only to malaria and, in terms of disability-adjusted life years (DALYs), the third-most common cause of morbidity after malaria and schistosomiasis, with children under 15 years of age suffering most of the disease burden (Daniella, 2015). The number of cases of visceral leishmaniasis (VL) is

calculated to be as high as 0.2–0.4 million people per year, approximately 90% of all these are found in three areas: the drainage basin of the Ganges river in eastern India and neighboring areas of southern Nepal (Terai) and areas of Bangladesh that share the same ecology; the Sudan, where a large epidemic has occurred among displaced people (Alvar *et al.*, 2012).

Cutaneous leishmaniasis (CL) has been considered by the World Health Organization (WHO) a category 1 emerging and uncontrolled disease (De Vries *et al.*, 2015), approximately 90% of the world's CL cases occur in Iran, Saudi Arabia, and Syria in the Middle East; in Afghanistan in Central Asia; and in Brazil and Peru in Latin America (Desjeux, 2004). Approximately 35,000 cases of mucosal leishmaniasis occur annually, mainly in Brazil, Peru, and Bolivia (Pace, 2014). VL is hypo endemic in Mediterranean countries; cases in this area account for 5–6% of the global burden (Daniella, 2015). It does not necessarily lead to clinical disease; most infections remain asymptomatic, but malnutrition and

immune suppression, notably HIV, predispose to clinical diseases (Okwor and Uzonna, 2013). VL has also emerged as an important opportunistic disease in persons with acquired immunodeficiency syndrome (AIDS) in the Mediterranean region, mainly in France, Italy, Portugal, and Spain (Monge-Maillo *et al.*, 2014). Since highly active antiretroviral therapy was introduced in 1997, a marked decrease in the number of co-infected cases both in these regions and in India have been reported (Singh, 2014). Persons who have had organ transplants, and in association with other conditions in which cell-mediated immunity is compromised, VL presents as opportunistic disease. In a multicenter study by Clemente, it was observed that diagnosis was frequently delayed and relapses were common after treatment (Clemente *et al.*, 2015). This disease remains a major neglected tropical disease, and a strategic framework for its control in the WHO European region has been applied (World Health Organization, Regional Office for Europe, 2014).

## LIFE CYCLE

These intracellular protozoa have a complex digenetic life cycle, requiring a susceptible vertebrate host and a permissive insect vector, which allow their transmission. Female sand flies of the genus *Lutzomyia* in the Americas and *Phlebotomus* elsewhere transmit *Leishmania* spp. (Lewis, 1987). The sand flies are modified pool feeders, meaning that they feed on pooled blood from their bite. Adults, males and females, are recognized by their small size, hairy body, the wings held erect on the body when resting, and a bouncy flight. Females are blood feeders and, in general, tend to bite at night or in twilight, but also can sting during the day in forested areas (Brazil *et al.*, 2015).

## MODE OF TRANSMISSION

Bite of an infected sandfly mainly during the late evening or the night time. Minimum 10–1,000 promastigotes per infective bite are required to initiate the infection (Apurba *et al.*, 2014).

## VERTEBRATE HOSTS, INCLUDING HUMANS

Promastigotes are regurgitated from the midgut rarely or directly discharged from foregut (proboscis) of the female sandfly into the skin of the vertebrate host. Promastigotes are phagocytosed by the skin macrophages and transform into amastigote forms within 12–24 h. The amastigote forms inside the macrophages multiply further

causing cell rupture and release into the circulation. Amastigotes are carried out in the circulation to various organs like liver, spleen and bone marrow and invade the reticuloendothelial cells like macrophages and endothelial cells (Apurba *et al.*, 2014).

## SANDFLY

During the blood meal taken up by the sandfly, the amastigotes are ingested and transformed into promastigote forms in the insect midgut. Promastigotes multiply by longitudinal fission and pass through various stages such as:

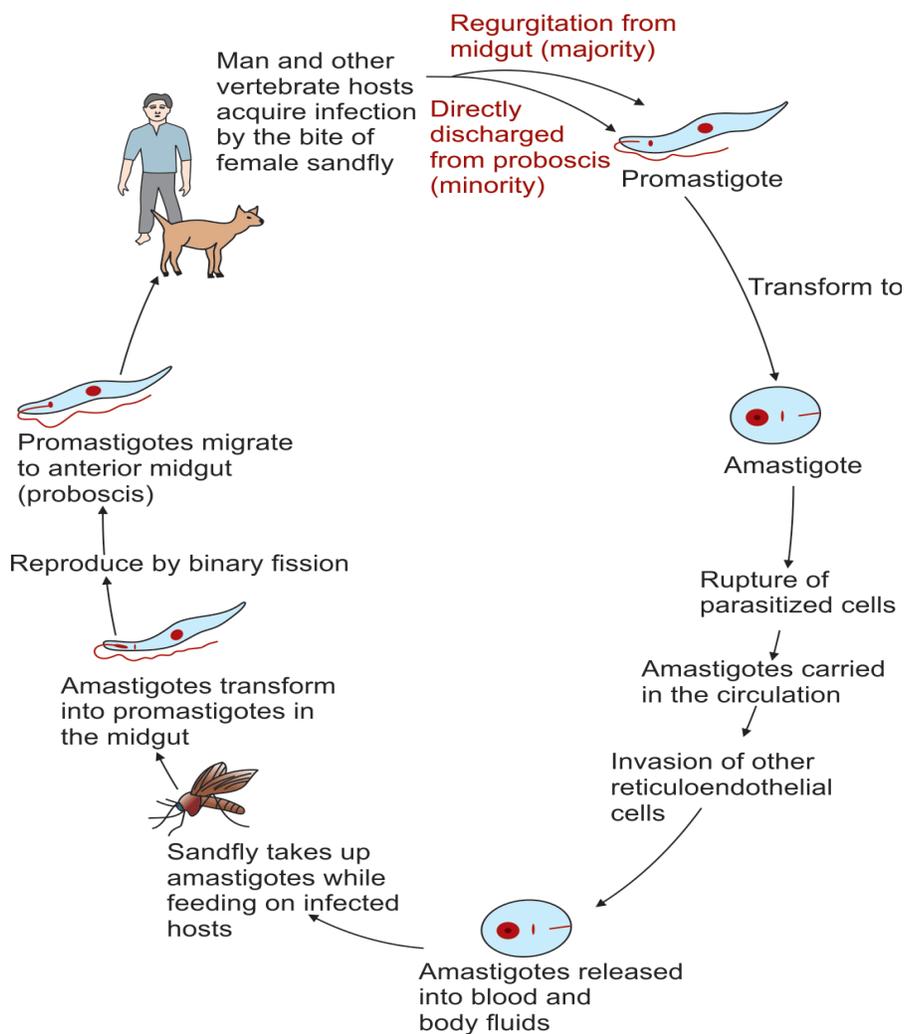
Amastigote → procyclic promastigote → nectomonad promastigote → haptomonad promastigote → leptomonad promastigote → metacyclic promastigote (Apurba and Sandhya, 2014). The metacyclic promastigotes multiply in the midgut of vector by binary fission and a small proportion migrate to the foregut (proboscis). They infect a new host during another blood meal. The duration of the life cycle in sandfly varies from 4 to 18 days depending on the species (Apurba and Sandhya, 2014) (Figure 1). Depending on the *Leishmania* sp., the sandfly genus, and the geographic location, the major reservoirs are canines, rodents, or humans (Andrade *et al.*, 2007; Bates, 2007). Although most transmission is by sandfly bites, *Leishmania* can be transmitted by blood transfusions, sharing of needles by intravenous drug abusers, occupational exposures, congenital transmission, and rarely by sexual transmission (Dey and Singh, 2006). Leukodepletion effectively reduces or eliminates transfusion-associated risk of *Leishmania* infection (Cruz *et al.*, 2002).

## CLASSIFICATION OF LEISHMANIASIS

*Leishmania* has two subgenera *L. Leishmania* and *L. Viannia*. The main difference between the two subgenera is that promastigotes of the subgenus *Viannia* develop in the midgut and hindgut of sandfly where as that of subgenus *Leishmania* develop in the anterior portion of the alimentary tract of sandfly. Both of the subgenera comprise of nearly 20 species (Abazid *et al.*, 2012); Old world leishmaniasis: Affects Asia, Africa and Europe and transmitted by sandfly (Genus *Phlebotomus*) New World Leishmaniasis: Affects Central and South America and transmitted by sandfly (Genus *Lutzomyia*) (Tables 1-3).

## CLINICAL SYNDROMES OF LEISHMANIASIS INCLUDE

*Visceral leishmaniasis (VL)*  
*Post-kala-azar dermal leishmaniasis (PKDL)*  
*Cutaneous leishmaniasis (CL)*  
*Diffuse cutaneous leishmaniasis (DCL)*



**Figure 1.** Sandfly mode of transmission of leishmaniasis.

*Leishmaniasis recidivans (LR)*

*Mucocutaneous leishmaniasis (MCL)*

### VISCERAL LEISHMANIASIS (VL)

Visceral leishmaniasis is mainly caused by the *L. donovani* and sometimes by *L. infantum*, (designated as *L. chagasi* in the New World) together known as *L. donovani* complex (Kuhls *et al.*, 2011). VL is a spectrum of symptoms and findings. At one extreme are persons with asymptomatic, in appearance, or self-resolving infections. At the other end are those with classic VL (kala-azar), with a characteristic pentad of prolonged fever, weight loss, hepatosplenomegaly, pancytopenia, and hypergammaglobulinemia. Ratios of asymptomatic

infection to VL cases are reported to range from 6.5: 1 in children to 18: 1 in adults with *L. infantum/L. chagasi* in northeastern Brazil (Evans *et al.*, 1992). The incubation period for full-blown visceral leishmaniasis is typically 3- 8 months, Persons who are immunocompromised by AIDS, neoplasm, or immunosuppressive therapy are at increased risk of asymptomatic parasitemia (Orsini *et al.*, 2012) and developing progressive disease. Classic visceral leishmaniasis, or kala-azar, is characterized by fever, malaise, weight loss, hepatomegaly, and splenomegaly.

The onset of symptoms is usually insidious, but on occasion it is abrupt and may suggest malaria or other acute infections. The fever may be intermittent, remittent with twice daily temperature spikes or less commonly,

Old world *Leishmaniasis*

**Table 1.** Leishmania Leishmania (L. L.) donovani complex

<b>Species</b>	<b>Geographical distribution</b>	<b>clinical syndrome</b>	<b>Vector (sandfly)</b>	<b>Reservoir</b>	<b>Transmission</b>
<i>L. L. donovani</i>	South Asia (Indian subcontinent)	VL (Kala-azar) PKDL	<i>Phlebotomus argentipes</i>	Humans	Anthroponotic
	Sudan, Ethiopia, Kenya and Uganda	VL, PKDL	<i>P. orientalis</i> , <i>P. martini</i>	Humans/ rodents	Anthroponotic/ Zoonotic
<i>L. L. infantum</i>	Middle East, Africa and China Mediterranean, Middle East, Central Asia and China	VL VL, CL	<i>P. perniciosus</i> <i>P. perniciosus</i>	Dogs, foxes, jackals	Zoonotic

**Table 2.** *L. L.* tropical complex

<i>L. L. tropica</i>	Western India, North Africa, Mediterranean, littoral, Middle East		CL, LR	<i>P. sergenti</i>	Humans	Anthroponotic
<i>L.L. aethiopica</i>	Ethiopia, Uganda, and Kenya		CL, DCL	<i>P. longipes</i>	Hyraxes	Zoonotic
<i>L. L. major</i>	Middle East, India, China Africa, central and western Asia		CL	<i>P. papatasi</i>	Rodents	Zoonotic

**Table 3.** New world *Leishmaniasis*

<i>L. L. chagasi</i> (new world variant of <i>L.L. infantum</i> )	Central and South America	VL, CL	<i>Lutzomyia</i> spp.	Dogs Foxes	Zoonotic
<i>L. L. Mexicana</i> Complex	Central America and northern parts of South America	CL, DCL	<i>Lutzomyia</i> spp.	Forest rodents	Zoonotic
<i>L. Viannia braziliensis</i> Complex	South and Central America	CL, MCL	<i>Lutzomyia</i> spp.	Forest rodents	Zoonotic

**Abbreviations:** VL, Visceral leishmaniasis; PKDL, post-kala-azar dermal leishmaniasis; CL, cutaneous leishmaniasis; LR, leishmaniasis recidivans DCL, diff use cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis.

and continuous. On physical examination, the spleen is firm, non tender, and frequently massively enlarged.

### VISCERAL LEISHMANIASIS IN PATIENTS WITH AIDS

Persons with concurrent visceral leishmaniasis and HIV infection present in a classic manner with fever, weight loss, and organomegaly and pancytopenia when patients have CD4+ counts greater than 50 cells/mm<sup>3</sup>, but atypical presentations and localization of parasites are more common when CD4 cells are less than 50 cells/mm<sup>3</sup> (Rosenthal *et al.*, 2000). AIDS patients have extensive gastrointestinal tract involvement that includes oral mucosa, esophagus, stomach, and small intestine and may present with chronic diarrhea.

### VISCEROTROPIC LEISHMANIASIS

This is a systemic syndrome characterized by chronic low-grade fever, malaise, fatigue, and in some instances,

diarrhea associated with *L. tropica* infection seen among U.S. military personnel who served in the Persian Gulf War of 1990 to 1991 (Magill *et al.*, 1994). Leishmania were isolated from the bone marrow specimens of these troops, who did not develop massive splenomegaly, wasting, or the progressive deterioration associated with classic kala-azar.

### Post-kala-azar dermal Leishmaniasis

It is a nonulcerative lesion of skin occurs in 2–50% of patients of VL following the completion of treatment. It is mainly seen in India and East African countries. PKDL is more commonly seen after treatment with pentavalent antimony (SbV) compounds and appears to be less common after successful treatment with amphotericin B formulations (Thakur *et al.*, 2008). The skin lesions of PKDL are chronic and may persist for as long as 20 years in India, whereas in the Sudan, they persist for only a few months to a year. The skin lesions vary from hyper pigmented or hypo pigmented macules progressing to

papules, nodules, and verrucous forms (Sultana *et al.*, 2012).

## CUTANEOUS LEISHMANIASIS

The spectrum of disease ranges from single, chronic ulcerative lesions (often referred to as "oriental sores") to disseminated, nonulcerative nodular lesions (a rare syndrome known as diffuse cutaneous leishmaniasis). Cutaneous leishmaniasis (CL) has been endemic in Aleppo, Syrian Arab Republic for centuries, giving the disease one of its common names, Aleppo boil (Canaan, 1929).

New World CL is endemic in widespread areas of Latin America. The causative species include *L. braziliensis*, *L. Mexicana*, *L. panamensis* and *L. Guyanensis*. Old World cutaneous leishmaniasis in the Mediterranean basin, the Middle East, southern Asia, India, and Africa are caused by three *Leishmania* species: *L. major*, *L. tropica*, and *L. aethiopica* (Alan, 2015). At the initial bite site, a papule forms, enlarges to a papulonodule, usually develops central ulceration, and slowly enlarges. Surrounding duration and raised borders are typical (Herwaldt *et al.*, 1992). CL is characterized by a mixed acute and chronic inflammatory infiltrate with infected and non infected mononuclear phagocytes, lymphocytes, and plasma cells. There are areas of focal necrosis (Hai *et al.*, 2004).

Leishmaniasis simulate a polar disorder similar to other intracellular infections such as leprosy (Alan, 2015; Barral *et al.*, 2004), ML, LR LCL DCL. For example, the range of clinical features in CL parallels that of leprosy. At the polyparasitic end of the spectrum is diffuse cutaneous leishmaniasis (DCL), a relatively uncommon syndrome, in which there is little evidence of effective cell-mediated immune response. Peripheral blood mononuclear cells neither proliferate nor produce interferon- $\gamma$  (IFN- $\gamma$ ) nor interleukin-2 (IL-2) in response to leishmanial antigens in vitro and cutaneous delayed-type hypersensitivity (DTH) reactions are absent (Silveira *et al.*, 2004). DCL has been compared with lepromatous leprosy, in which there is a large number of mycobacteria in macrophages and no evidence of protective, Th1 cell-mediated immune responses. At the oligoparasitic end of the spectrum is leishmaniasis recidivans (LR), a hyperergic variant of CL caused by *L. tropica* infection in the Old World, in which chronic lesions slowly expand while healing at the center. Amastigotes are sparse, and a mononuclear cell infiltrate predominates. This is somewhat analogous to tubercloid leprosy, in which there is an intense mononuclear infiltrate with few mycobacteria.

Knowledge of immunology of these parasites is important to ascertain the clinical spectrum of the disease. Oligoparasitic, such as ML and LR, are characterized by very few recognizable parasites, an exaggerated cell mediated immune response, and a minimal antibody response.

A review concerning outbreaks in Israel over a recent 13-year period revealed an expansion of CL illness in southern, central, and northern areas of the country (Gandacu *et al.*, 2014). Therefore, studies of the responsible factors and a greater degree of public alertness are necessary (Daniella, 2015).

## DIAGNOSIS

The diagnosis of leishmaniasis is frequently delayed and may be missed. Clinical diagnosis of VL has a pentad of prolonged fever, progressive weight loss, pronounced hepatosplenomegaly, pancytopenia and hypergammaglobulinemia from a known endemic area (Pace, 2014). Several methods of laboratory diagnosis of leishmaniasis have been reported (Elmahallawy *et al.*, 2014), these include parasite detection by microscopic examination, culture and successive isoenzyme analysis for identification, or molecular biology-based assays for detecting the parasite DNA (polymerase chain reaction [PCR]). The diagnosis may be difficult in patients with late-stage HIV infection or AIDS and returning travelers who present with atypical signs and symptoms. The confirmation of diagnosis is done by parasitologic demonstration of amastigotes in tissue, by isolating promastigotes in culture, or with a positive PCR assay (Alan, 2015). Splenic aspiration, liver biopsy, lymph node aspirates, and bone marrow aspirates have all been used with success (Alan, 2015). The spleen is the most sensitive location, the sensitivity of a bone marrow aspirate approaches that of a splenic aspirate when microscopists spend more time reviewing the smear (Sarker *et al.*, 2004). Bone marrow aspiration is safer and preferred in non-endemic settings but is less sensitive. Amastigotes may also be seen within mononuclear cells in Wright- and Giemsa stained smears of the buffy coat. Aspirates from the spleen, bone marrow, liver, or lymph node can be cultured. Specimens can be inoculated into media such as Schneider's modified or Novy-MacNeal-Nicolle [NNN] and maintained at ambient temperatures 22-26°C.

Enzyme-linked immunosorbent assay and dipstick tests using *L. infantum/L. chagasi* recombinant k39 (rk39), a kinesin-like antigen (Burns *et al.*, 1993) have demonstrated excellent sensitivity and specificity for the diagnosis of VL in immuno competent persons in India and Brazil but less so in East Africa, (Ritmeijer *et al.*, 2006; Chappuis *et al.*, 2006; Braz *et al.*, 2002).

Cutaneous leishmaniasis, a definite diagnosis is made by identifying amastigotes in tissue, promastigotes in culture, or by amplifying *Leishmania*-specific DNA or RNA in a PCR assay. Specimen can be obtained from an ulcer base after meticulous and thorough cleaning and removal of exudate before the scraping, aspiration, or biopsy. Scrapings are often successful in confirming the diagnosis. Culture and PCR assays are also done for

confirmation of the diagnosis (Alan, 2015). The biopsy specimen should be divided and used for culture, touch preparation, and histopathology. Serologic assays are not generally helpful in the diagnosis of CL. A positive serological test for leishmanial antibodies provides only presumptive evidence of infection because cross-reacting antibodies may be present in persons with Chagas' disease or leprosy.

## TREATMENT

*Leishmania* infections have genetic heterogeneity with each region having different species complexes. These differences may be reflected in variable natural history of infection and response to treatment. Different sandfly vectors, mammalian reservoirs, and human hosts with different genetic backgrounds could also be responsible in variable response to treatment (Alan, 2015). The drugs employed to treat VL are expensive and sometimes have toxic side effects (Daniella, 2015). Single dose liposomal amphotericin B (AmBisome; Astellas, Northbrook, IL) leads to greater than 95% efficacy in India (Mueller *et al.*, 2007) but requires a much higher total dose in the Sudan (Mueller *et al.*, 2006; Sundar *et al.*, 2014).

Paromomycin, an aminoglycoside antibiotic, is used for the treatment of both VL and CL in parenteral and topic formulations. Open-label study evaluated the efficacy of two treatment options, single infusion of preformed amphotericin B (AmB) lipid and injectable Paromomycin, an aminoglycoside (Sundar *et al.*, 2007). The Paromomycin was shown to be non-inferior to amphotericin. Miltefosine was registered in India in 2002 as the first oral antileishmanial agent; it is a phosphocholine analogue, its major limitations are the high cost, the need for monitoring of gastrointestinal side-effects, and occasional hepatic and renal toxicity (Sundar *et al.*, 2012). Combining Paromomycin with miltefosine, the release of nitric oxide and tumor necrosis factor by human macrophages was induced, enhancing the killing of *L. donovani* promastigotes *in vitro* (Das *et al.*, 2012).

Pentavalent antimony, multiple preparations of this drug exist; Sodium stibogluconate (SSG; Pentostam, Glaxo Smith-Kline, Brantford, Middlesex, England), and meglumine antimoniate (MA; Glucantime, Sanofi-Aventis, Paris) are the two best known commercial products. SbV is still used for the treatment of VL in areas where *Leishmania* isolates remain susceptible. In many cases treatment failure is due to abnormalities in the host's immune system or sometimes attributable to drug resistance in the parasite, in this situation liposomal amphotericin B, amphotericin B deoxycholate, or miltefosine can be used. Primary failures and relapses are often observed in patients with concurrent AIDS (Alan, 2005). For cutaneous lesions especially in cutaneous leishmaniasis, several drugs have been used;

these include Sodium stibogluconate Amphotericin B and pentamidine. Local injections of SbV have been used successfully in some settings. Oral systemic treatment in Old World CL with several agents has been studied. Fluconazole (Alrajhi, 2002) and Itraconazole (Nassiri-Kashani *et al.*, 2005) all appear to have activity against some *Leishmania* species. In all forms of CL, attention should be directed to local wound care. CL is both a parasitic infection and an open wound. Antibiotics should be administered if there is evidence of cellulitis, painful or tender areas, or purulence.

## PREVENTION

Standard personal protective measures, such as *N, N*-diethyl-meta-toluidine (DEET)-based insect repellents and permethrin or other insecticides applied to clothing and insecticide impregnated fine-mesh bed nets all provide protection against sand flies if used correctly (Soto *et al.*, 2006). Vector control and reservoir control are effective. Although no commercial vaccine is currently available, there is a rationale to expect one in the future. The spontaneous resolution of human infection is associated with high-level immunity against the homologous infecting *Leishmania* spp. *Phlebotomus* are not known to fly high above the ground level and it is nocturnal in habitat. So, sleeping at top floors or on the beds high above the ground can prevent transmission. Early treatment of all cases is of paramount importance.

## CONCLUSION

Leishmaniasis is not only a neglected tropical disease, it is also a major health problem worldwide. It produces varying group of clinical syndromes ranging from self-healing cutaneous ulcers (in apparent) to fatal visceral disease. It is mainly a zoonotic disease affecting dogs, foxes, jackals and rodents. Animal reservoir plays a major role for transmission. Lot of risk factors associated with *Leishmania* distribution have been implicated; these include, socioeconomic conditions, malnutrition, population mobility, and environmental and climate changes. Prevention and control are based on early diagnosis and treatment, vector control, disease surveillance, and education of the community (Daniella, 2005). It is second protozoan infection of medical importance to for human health after malaria, Parasitological diagnosis is made on the basis of biopsy results, and treatment requires drugs that are toxic and expensive. Many of these drugs are still out of reach. Spraying of houses with residual insecticide is difficult to sustain, as is control of animal reservoirs (e.g., dogs, for VL). Recent research has resulted in the development of insecticide, treated bed nets, miltefosine, and cheap and

reliable serological tests for leishmaniasis (Guerin *et al.*, 2002; Davies *et al.*, 2003).

## Dedication

This article is dedicated to Prof. Rasheed Bakare, a brilliant Medical Practitioner, teacher and mentor. Bakare is well-known as a visiting teacher in different prestigious institutions of West African countries and for various projects. His special interest in sexually transmitted diseases and infectious diseases has earned him respect among his colleagues. He has trained quite a number of Medical doctors, Nurses and Medical Laboratory Scientists both at undergraduate and postgraduate levels. I am proud to know him as a mentor, teacher and a senior professor in the field of Medical Microbiology and Parasitology.

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