Genus- *Leishmania*

Leishmania is a genus under the family Trypanosomatidae that are responsible for the disease leishmaniosis. They are spread by sandflies of the genus *Phlebotomus* in the Old World, and of the genus *Lutzomyia* in the New World. At least 93 sandfly species are proven or probable vectors worldwide. Their primary hosts are vertebrates; *Leishmania* commonly infects hyraxes, canids, rodents, and humans.

History

Leishmania amastigotes have been first observed by in 1885 by Cunningham in skin lesions of patients from India, but he suggested that they were members of *Mycetozoa* (fungi). Protozoal nature of *Leishmania* was first recognized in 1898 by Borovsky during his study of skin lesions in Turkmenistan. William Boog Leishman in 1903 discovered similar intracellular bodies in the visceral organs of fatal cases of kala-azar from India, and established that they were morphologically related to trypanosomes he termed the disease as 'Dum-dum fever'. A few weeks later, the Irish doctor Charles Donovan (1863–1951) who was professor of physiology at the Madras Medical College, published a paper reporting that he had found similar bodies in splenic samples taken during life and at autopsy from native Indian subjects with remittent fever and enlarged spleens. Meanwhile, the British medical doctor Ronald Ross (1857–1932), who was ordered by the Indian government in 1898 to investigate kala-azar, published a paper in November 1903 commenting on the discovery of the ovoid bodies found by Leishman and Donovan in spleen pulp of patients with chronic pyrexia and splenomegaly. He concluded that the ovoid bodies were not degenerated trypanosomes but a novel protozoan organism and that the clinical picture of the cases resembled that of kala-azar and by the end of 1904 the term *Leishmania donovani* was adopted.The causal relationship between *Leishmania* parasites and development of cutaneous lesions was confirmed in 1908 by Martsinovsky who self-infected himself with the parasite cultures.

Morphology

Leishmania species are unicellular eukaryotes having a well-defined nucleus and other cell organelles including kinetoplasts and flagella. Depending on the stage of their life cycle, they exist in two structural variants, as

1. The **amastigote** form is found in the mononuclear phagocytes and circulatory systems of humans. It is an intracellular and nonmotile form, being devoid of external flagella. The short flagellum is embedded at the anterior end without projecting out. It is oval in shape, and measures 3–6 μm in length and 1–3 μm in breadth. The kinetoplast and basal body lie towards the anterior end.

2. The **promastigote** form is found in the alimentary tract of sandflies. It is an extracellular and motile form. It is considerably larger and highly elongated, measuring 15-30 μm in length and 5 μm in width. It is spindle-shaped, tapering at both ends. A long flagellum (about the body length) is projected externally at the anterior end. The nucleus lies at the centre, and in front of it are the kinetoplast and the basal body.

Life cycle

Leishmania is transmitted by the bite of female phlebotomine sandflies. Sandflies become infected during blood meals on an infected host when they ingest macrophages infected with amastigotes. In the sandfly's midgut, the parasites differentiate into promastigotes , which multiply by binary fission and migrate to the proboscis and form a plug. The sandflies inject the infective stage, promastigotes, during blood meals. Promastigotes that reach the puncture wound are phagocytized by macrophages and transform into amastigotes. Amastigotes multiply again by binary fission in infected cells and affect different tissues, depending in part on the *Leishmania* species. This originates the clinical manifestations of leishmaniosis.

Epidemiology

The disease is widespread in the tropical and subtropical areas and found in 98 countries in Europe, Africa, Asia and America. However, over 90% of new cases occur in just 13 countries (Afghanistan, Algeria, Bangladesh, Bolivia, Brazil, Columbia, Ethiopia,

India, Iran, Peru, South Sudan, Sudan and Syria). It is estimated that between 0.9 and 1.7 million people are newly infected every year, but only a small fraction ofthem will develop the disease and 20,000–30,000 will eventually die. In India visceral leishmaniosis is prevalent in states of Assam, West Bengal, Bihar endemic foci reported from Tamil Nadu and sporadic cases from Gujrat, Uttar Pradesh, Punjab, foot hills of Himalayas, Jammu region and Pudduchery.

Although the different Leishmania species are morphologically very similar, they cause two main clinical forms, cutaneous leishmaniosis (CL) and visceral leishmaniosis (VL), depending on which types of phagocytic cells are invaded. In CL, the parasites infect macrophages resident in the skin. When the host cell is full of parasites, it bursts and the released amastigotes will infect neighboring macrophages. In VL, however, the released amastigotes are spread by the blood circulation and infect cells of the mononuclear phagocyte system (reticulo-endothelial system) of liver, spleen, bone marrow, lymph nodes and the intestine.

Visceral Leishmaniosis

Important members which are involved in visceral leishmaniosis are ***Leishmania donovani, L. infantum, L. chagasi***

Leishmania donovani causeskala-azar, dum-dum fever, black fever or visceral leishmaniosis in humans.

As per the place where this infection takes place visceral leishmaniosis or kala-azar can be classified as Indian kala-azar, Chinese kala-azar, Mediterranean (infantile) kala-azar, African kala-azar, Russian kala-azar and American kala-azar.

There are three ecological forms of *Leishmania donovani*

a. *L. donovani donovani* of Indian subcontinent and Burma largely Assam and Bengal. Affecting young adults of 10-20 years. Dogs are not reservoir host and transmitted by *Phlebotomus argentipes.*
b. *L. donovani sensulato* of Kenya, southern Ethopia, Somalia and Uganda. It occurs in young adult and is anthroponotic with involvement of rodent (ground-squirrelas reservoirs. It is transmitted by *P. vansomerenae* and *P. celiae*

c. *L. donovani sensulato* of Sudan, western Ethopia, Chad, Niger etc. it is a zoonotic and carnivores and rodents

Pathogenesis-

- It is a fatal disease
- The primary cell types Leishmania infiltrates are phagocytotic cells such as neutrophils and macrophages. These are called as 'leishman Donovan' (LD) bodies. After a period of weeks or months they invade the inner organs to multiply in spleen, liver, bone marrow and elsewhere.
- Phagocytotic immune cell like a macrophage will ingest a pathogen within an enclosed endosome and then fill this endosome with enzymes which digest the pathogen. However, in the case of *Leishmania* spp, these enzymes have no effect, allowing the parasite to multiply rapidly. This uninhibited growth of parasites eventually overwhelms the host macrophage or other immune cell, causing it to die.

Clinical Signs/Symptoms-

The stereotypical manifestations of clinically manifest visceral infection include

- Fever
- Weight loss
- Hepatosplenomegaly (usually, the spleen is more prominent than the liver)
- Pancytopenia—i.e., anemia, leukopenia, and thrombocytopenia
- A high total protein level and a low albumin level, with hyper gamma globulinemia and Lymphadenopathy

  Post kala-azar dermal leishmaniosis (PKDL)-Some patients develop a syndrome characterized by skin lesions (such as erythematous or hypopigmented macules, papules, nodules, and patches), typically first noticed and most prominent on the face, that develop at variable intervals (2-10 years) after (or during) therapy for visceral leishmaniosis. Persons with chronic PKDL can serve as important reservoir hosts of infection. These individual are immune against the infection.

Diagnosis-

- Symptoms as mentioned above

- Post-mortem findings like enlargement of spleen, liver and lymph node etcanaemia and emaciation
- Microscopic examination amastigotes stage is seen upon microscopic examination of tissue specimens. Amastigotes can be visualized with both Giemsa and hematoxylin and eosin (H&E) stains.
- Culture and isolation Isolation can be done using the biphasic medium which includes a solid phase composed of blood agar base (e.g., NNN medium), with defribinated rabbit blood.
- Isozyme analysis- After isolation parasites can be characterized to the complex and sometimes to the species level using isoenzyme analysis, which is the conventional diagnostic approach for Leishmania species identification.
- Serological tests- Antibody detection can prove useful in visceral leishmaniosis. Diagnostic Tests like CFT, IHA, IFA, ELISA etc, are sensitive and specific. Non-specfic tests like formal gel test and urea Stilbamide test have been done in India and China
- Molecular approaches have the potential to be more sensitive and rapid; e.g., the results can be available within days versus weeks. The method is based on PCR amplification using generic primers that amplify a segment of the rRNA internal transcribed spacer 2 (ITS2) from multiple Leishmania species.

Treatment

- The drugs used to treat both visceral and cutaneous leishmaniasis are pentavalent antimonials (sodium stibogluconate @ 2-5ml i/m on alternate day 10 injection, Ethyl estilbamine @ 0.1-0.2 gm/day total 2.7 gm and meglumine antimoniate),
- amphothericin B @5-10 mg/kg bwt i/v
- pentamidine. @4 mg/kg bwt i/m
- A range of alternative drugs including miltefosine, paromomycin, azoles, azithromycin, allopurinol, dapsone and rifampicin have been recently introduced.

Control

- Treatment of infected host
- Control of vectors using insecticides and repellants, destroying of breeding places of the fly, clearing of decaying matter and vegetation

Other members causing visceral leishmaniosis are

- *L. infantum* causing Chinese kala-azar, Mediterranean kala-azar and Russian kala-azar it causes disease mainly in children mainly under five years with dog as reservoir host and clinical host. It is transmitted by *P. chinensis.*
- *L. chagasi* causing New World visceral leishmaniosis or American kala-azar. It occur in humans of all ages and in canids. Transmission takes place by *Lutzomiya* spp.

Cutaneous leismaniosis

Cutaneous leishmaniosis is the most common form of leishmaniosis affecting humans and canids. It is a zoonotic disease. It is a skin infection caused by a single-celled parasite that is transmitted by the bite of a phlebotomine sandfly. There are about twenty species of *Leishmania* that may cause cutaneous leishmaniosis. In the Old World, CL is known as Oriental sore, Aleppoboil, Jeriho boil, Baghdad boil, Balkh sore, Penjdeh sore, Briska button (clou de Briska), Bouton de Crete and BoutonD'Orient. In the New World, the disease is known as Uta, Espundia, Chiclero'e ulcer, Pain bois and forest yaws.

Cutaneous form of the disease is caused mainly by *L. tropica, L. aethiopica* and *L. major* in the Old World, and by *L. braziliensis*, *L. guyanensis*, *L. panamensis,* L. peruviana, *L. mexicana*, *L. amazonensis,* and *L. venezuelensis* in the New World.

Host- Human, dog and wild animals are usual host. Other rodents like gebrils, rats are important reservoir host.

Epidemiology-Cutaneous leishmaniosis is endemic in more than 70 countries, with an estimation of 1.5-2 million new cases every year. Afghanistan, Syria, and Brazil are the main foci. In India it is found in Rajasthan, Punjab, Delhi, Haryana and Gujrat etc.

Pathogenesis and clinical signs- In CL, the parasites infect macrophages resident in the skin. When the host cell is full of parasites, it bursts and the released amastigotes will infect neighbouring macrophages. Cutaneous leishmaniasis (CL) in humans often involves only the skin, without mucosal or visceral involvement. Initially, one to multiple erythematous papules, which may sometimes be pruritic, appear on the skin. These papules can develop into ulcers, which typically have raised, indurated margins; nodules, which may be smooth or covered in scales; flat plaques; or hyperkeratotic wart-like

lesions. *L. major* lesions tend to be exudative or "wet," and prone to secondary bacterial infections, while *L. tropica* infections tend to be "dry," with a central crust. Skin lesions may be accompanied by regional lymphadenopathy, which occasionally persists after the lesions have healed. Peripheral neuropathy has also been reported. Many cases of cutaneous leishmaniosis remain localized; however, secondary lesions sometimes appear on the skin, or occasionally the mucosa, in other parts of the body.

The dermal lesions associated with cutaneous leishmaniosis in dogs include exfoliative dermatitis, which can be generalized or localized over the face, ears, and limbs. Ulcerative, nodular, or mucocutaneous dermatitis are also seen. Cutaneous ulcers over the ears or other locations may be associated with considerable bleeding.

Cats- In cats, skin lesions tend to occur on the nose, ears, eyelids or lips, but they can also be found on other sites such as the paws. Localized nodules, papules and chronic crusted or ulcerated lesions are seen most often, and may be accompanied by regional lymphadenopathy. Alopecia, scales and hemorrhagic pustules or nodules have been reported infrequently.

Ruminants- Clinical cases have rarely been described in cattle or small ruminants. Skin lesions, sometimes accompanied by lymphadenopathy, were the only clinical signs reported in sheep, a goat and cattle.

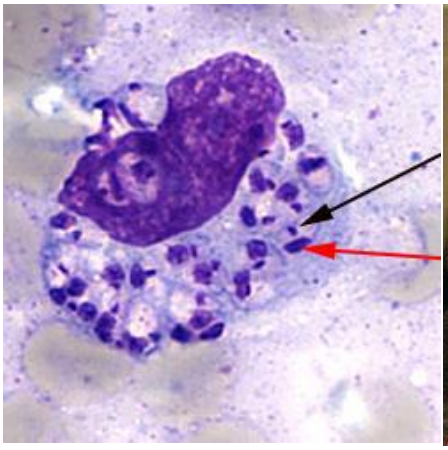
Equines- Horses, mules and donkeys sometimes develop skin lesions, particularly on the head, neck, legs and axillary or inguinal regions. The most common lesions are solitary or multiple papules or nodules, which are often ulcerated. Disseminated skin disease has also been reported.

Diagnosis- Cutaneous leishmaniosis can be diagnosed by direct observation of the parasites under microscope.Various quantitative serologic methods to detect anti-Leishmania antibodies have been developed, including indirect immunofluorescence assays, ELISA, and direct agglutination assays. Immunohistochemistry or culture in NNN medium, or in animals.Amastigotes are easiest to detect visually in recent or active lesions or in cases of diffuse cutaneous leishmaniasis. A delayed hypersensitivity test, the leishmanin skin test (Montenegro skin test), may be useful in the diagnosis of cutaneous

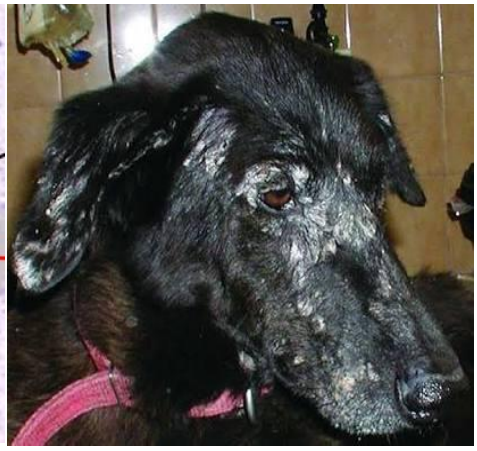
and mucocutaneousleishmaniasis, especially outside endemic areas.Detection of parasite-specific DNA by PCR allows sensitive and specific diagnosis of infection.

Treatment and control- Pentavalent antimonials (e.g., sodium stibogluconate, meglumine antimoniate) can be used to treat leishmaniosis where the parasites are sensitive to these drugs other drugs used in viscera leishmaniosis can be used.

Keeping susceptible animals, indoors between dusk and dawn, especially during the warmer months, can reduce their exposure to sandflies. Insecticide-impregnated collars or topical insecticides (spot-on preparations, sprays) are reported to decrease sandfly bites in dogs.

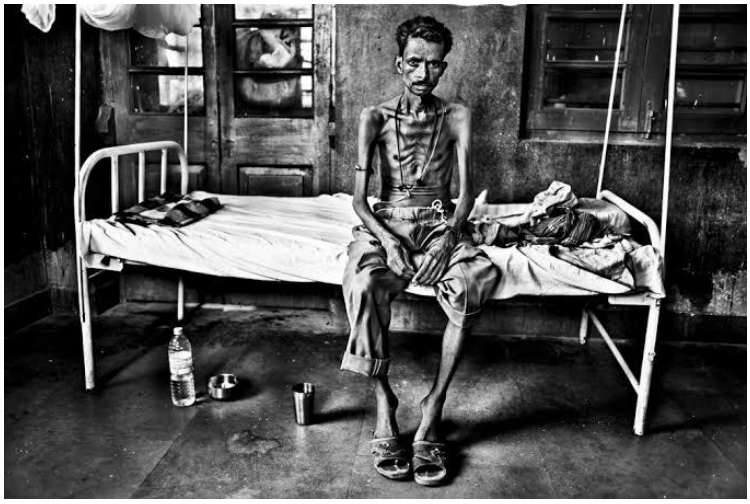


Amastigote stage in a macrophage



A dog having Cutaneous Leismaniosis

A man showing Cutaneous Leismaniosis



A patient suffering from black fever (Kala-azar)

(Coursy: The photographs has been taken from internet)