## Lecture 5 Hemoflagellates

Flagellates found in blood and tissues.

## Leishmania Donovani

*L. donovani* causes **visceral leishmaniasis** or **Kala-azar**. It also causes the condition, **Post Kala-azar Dermal Leishmaniasis (PKDL)**.

### History and Distribution

Sir William Leishman in 1900, observed the parasite in spleen smears of a soldier who died of '**Dumdum fever**' or Kalaazar contracted at Dum Dum, Calcutta. Leishman reported this fi nding from London in 1903. In the same year, Donovan also reported the same parasite in spleen smears of patients from Madras. The name *Leishmania donovani* was, therefore given to this parasite. The amastigote forms of the parasite as seen in smears from patients are called **Leishman Donovan** (LD) bodies.

***Visceral leishmaniasis or Kalaazar**

Is a major public health problem in many parts of world. According to the World Health Organization (WHO), a total of 5,00,000 cases of visceral leishmaniasis occur every year. Of these new cases, 90% are found in the Indian subcontinent and Sudan and Brazil. The disease occurs in endemic, epidemic, or sporadic forms. Major epidemics of the disease are currently found in India, Brazil, and Sudan.

*India, beginning in the mid1970s, assumed epidemic proportions in 1977 and involved over 1,10,000 cases in humans. Initially, the disease was confi ned to Bihar (Muzaff arpur, Samastipur, Vaishali, and Sitamarhi). Since then, the cases are increasing and involving newer areas. The epidemic extended to West Bengal and fi rst outbreak occurred in 1980 in Malda district.

*At present, the disease has established its endemicity in 31 districts in Bihar, 11 districts in West Bengal, 5 districts in Jharkhand, and 3 districts in Uttar Pardesh. Sporadic cases have been reported from Tamil Nadu, Maharashtra, Karnataka, and Andhra Pradesh.

### Habitat

The amastigote (LD body) of *L. donovani* is found in the reticuloendothelial system. They are found mostly within the macrophages in the spleen, liver, bone marrow and less often in other locations such as skin, intestinal mucosa, and mesenteric lymph nodes.

### Morphology

The parasite exists in 2 forms  mammals.

***Promastigote form:** in the sandfl y and in artifi cial culture.

**Amastigote**

The amastigote form (LD body) is an ovoid or rounded cell, about 2–4 μm in size.

*It is typically intracellular, being found inside macrophages, monocytes, neutrophils, or endothelial cells.

*They are also known as **LD bodies**.

*Smears stained with Leishman, Giemsa, or Wright's stain show a pale blue cytoplasm enclosed by a limiting membrane.

*The large oval nucleus is stained red. Lying at the right angles to nucleus, is the red or purple stained **kinetoplast**.

*In well stained preparations, the kineloplast can be seen consisting of a **parabasal body** and a dotlike **blepharoplast** with a delicate thread connecting the two. The axoneme arising from the blepharoplast extends to the anterior tip of the cell.

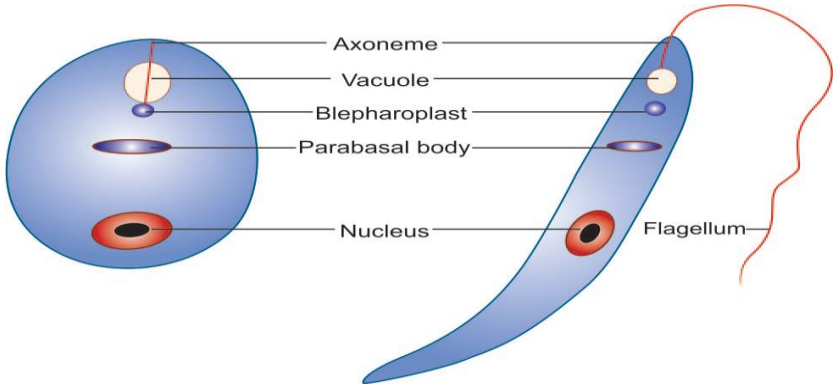
*Alongside the kinetoplast a clear unstained vacuole can be seen.

*Flagellum is absent. *Promastigote*. It is a fl agellar stage and is present in insect vector, sand fly and in cultures.

*The promastigotes, which are initially short, oval or pear-shaped forms, subsequently become long spindleshaped cells, 15–25 μm in length and 1.5–3.5 μm in breadth. A single nucleus is situated at the center. The kinetoplast lies transversely near the anterior end.

*The flagellum is single, delicate, and measures 15–28 μm.

*Giemsa or Leishman stained films show pale blue cytoplasm with a pink nucleus and bright red kinetoplast.




**Morphology of *Leishmania donovani*. A. Amastigote (LD body) ; B.Promastigote**

## Life Cycle

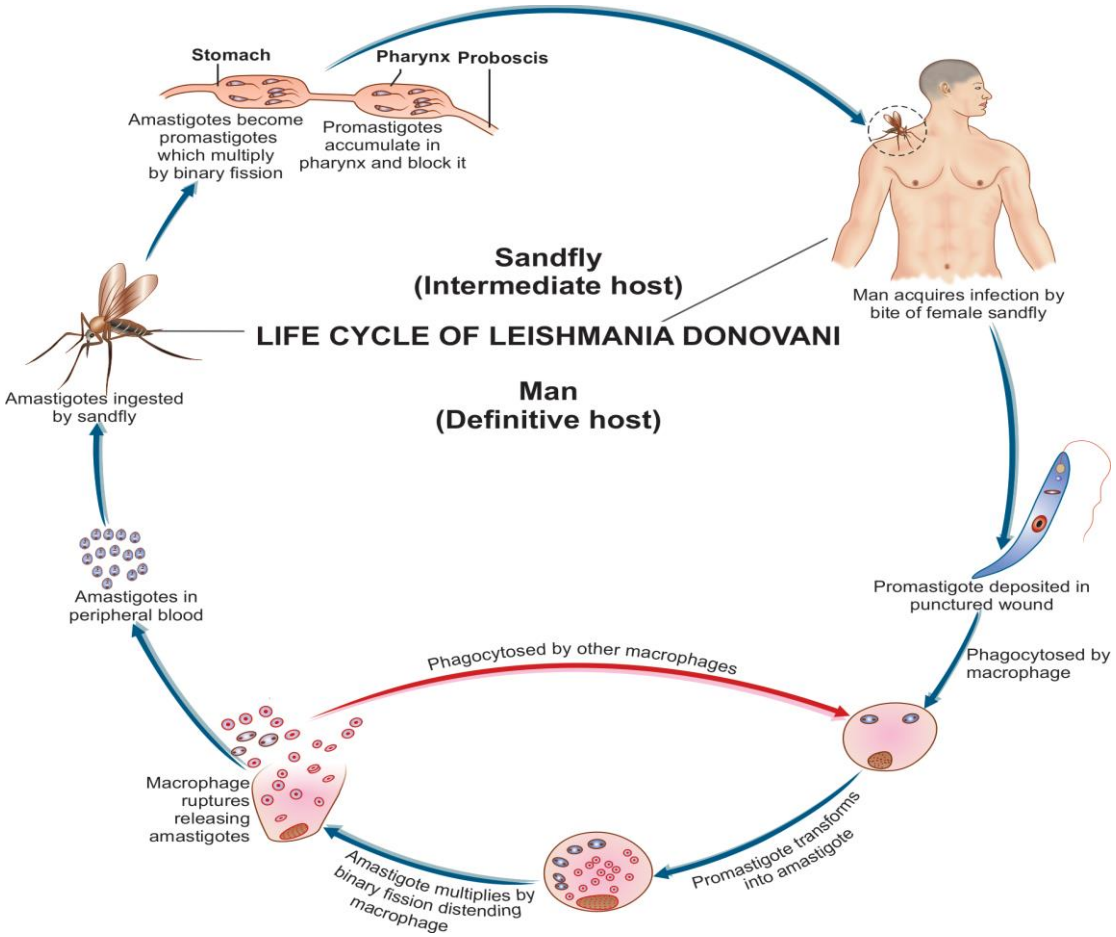
*L. donovani* completes its life cycle in 2 hosts. **Definitive host:** Man, dog, and other mammals.
**Vector:** Female sandfly (*Phlebotomus* species).
**Infective form:** Promastigote form present in midgut of female sandfly.

**Mode of transmission:**

 Humans acquire by bite of an infected female sandfly. It can also be transmitted vertically from mother to fetus, by blood transfusion, and accidental inoculation in the laboratory.
**Incubation period:** Usually 2–6 months, occasionally it may be as short as 10 days or as long as 2 years.
*The sandfly regurgitates the promastigotes in the wound caused by its proboscis.
*These are engulfed by the cells of reticuloendothelial system (macrophages, monocytes, and polymorphonuclear leucocytes) and change into amastigote (LD body) within the cells.
*The amastigote multiplies by binary fi ssion, producing numerous daughter cells that distend the macrophage and rupture it. The liberated daughter cells are in turn, phagocytosed by other macrophages and histiocytes. Small number of LD bodies can be found in peripheral
blood inside neutrophils or monocytes.
*When a vector sandfl y feeds on an infected person, the amastigotes present in peripheral blood and tissue fluids enter the insect along with its blood meal. In the midgut (stomach) of the sandfl y, the amastigote elongates and develops into the promastigote form.
*The promastigote multiples by longitudinal binary fission and reaches enormous numbers. They may be seen as **large rosettes** with their flagella entangled.
*In the sandfl y, they migrate from the midgut to the pharynx and hypostome, where they accumulate and block the passage.
*Such **blocked sandflies** have difficulty in sucking blood. When they bite a person and attempt to suck blood, plugs of adherent parasites may get dislodged from wound. It takes about 10 days for the promastigotes to reach adequate numbers after ingestion of the amastigotes, so as to block the buccal cavity and pharynx of the sandfly. This is, therefore, the duration of

**Extrinsic incubation period**.

This period is also synchronous with the gonadotropic cycle of the vector, so that amastigotes ingested during a single blood meal, are ready to be transmitted when the sandfly takes the next blood meal after its eggs have been laid.




## Pathogenicity

*L. donovani* causes visceral leishmaniasis or kala-azar.

*Kala-azar is a reticuloendotheliosis resulting from the invasion of reticuloendothelial system by *L. donovani.*

*The parasitized macrophages disseminate the infection to all parts of the body.

*In the spleen, liver, and bone marrow particularly, the amastigotes multiply enormously in the fixed macrophages to produce a 'blockade' of the reticuloendothelial system. This leads to a marked proliferation and destruction of reticuloendothelial tissue in these organs.

***Spleen:**

. The spleen is the most affected organ. It is grossly enlarged and the capsule is thickened due to perisplenitis.

*__Liver:__

. The liver is enlarged.

. The Kupffer cells and vascular endothelial cells are heavily parasitized, but hepatocytes are not affected.

*__Bone marrow:__

The bone marrow is heavily infiltrated with parasitized macrophages, which may crowd the hematopoietic tissues.

**Peripheral lymphnodes** and lymphoid tissues of the nasopharynx and intestine are hypertrophic, although this is not seen in Indian cases.

*Severe **anemia** with hemoglobin levels of 5–10 g/dL may occur in Kala-azar, as a result of infiltration of the bone marrow as well as by the increased destruction of erythrocytes due to hypersplenism.

## Clinical Features of Kala-Azar

*The onset is typically insidious. The clinical illness begins with fever, which may be continuous, remittent, or irregular.

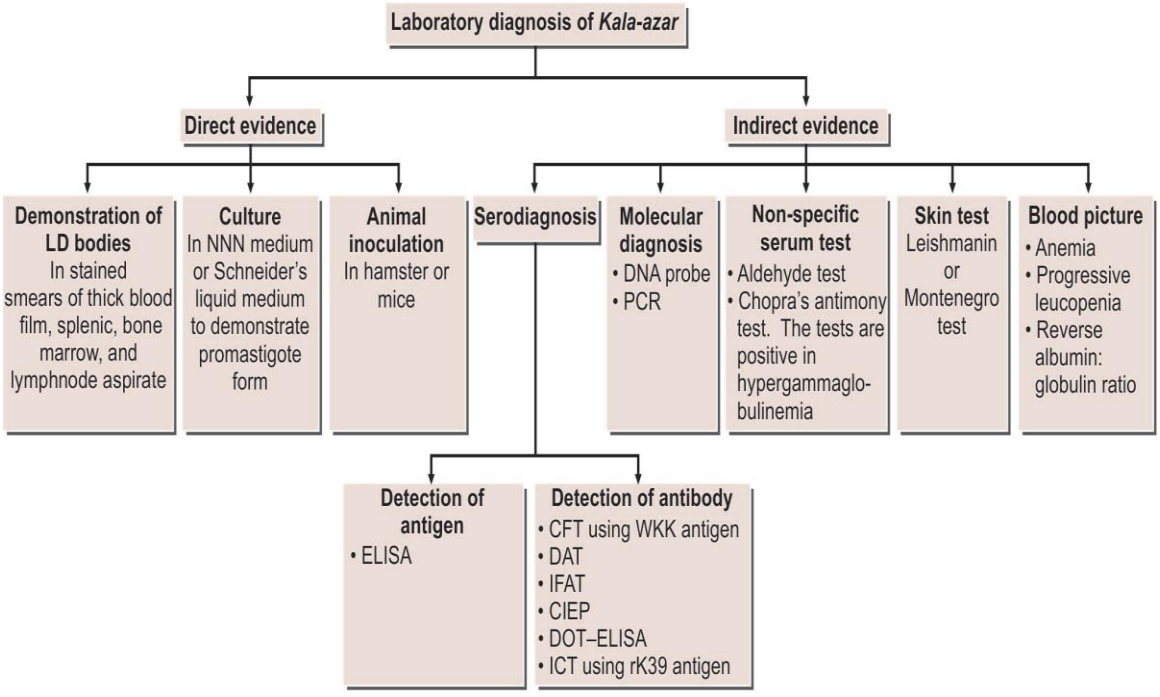
*__Splenomegaly__ starts early and is progressive and massive.

*__Hepatomegaly__ and __lymphadenopathy__ also occur but are not so prominent.

*Skin becomes **dry, rough,** and **darkly pigmented** (hence, the name **Kala-azar**).

## Post Kala-azar Dermal Leishmaniasis

About 3–10% cases of patients of visceral leishmaniasis in endemic areas develop PKDL, about a year or 2 after recovery شفاء from the systemic illness.



**Leishmania Tropica Complex**
It includes 3 species:
€ * *Leishmania tropica* * *Leishmania major* * *Leishmania aethiopica*
All these species cause old world cutaneous leishmaniasis. The disease is also known as **oriental sore**, **Delhi boil**, **Bagdad boil**, or **Aleppo button**.

**History and Distribution**
Cunnigham (1885) first observed the parasite in the tissues of a Delhi boil in Calcutta.

- Russian military surgeon, Borovsky (1891) gave an accurate description of its morphology and Luhe (1906) gave the name *L. tropica.*
- *L. tropica* and *L. major* are found in Middle-East, India, Afganistan, eastern Mediterranean countries, and North Africa.
- *L. aethiopica* occurs in Ethiopia and Kenya.

**Habitat**
*L. tropica* causing cutanaeous leishmaniasis (old world cutaneous leishmaniasis) are essentially the parasite of skin.
The amastigote forms occur in the reticuloendothelial cells of the skin, whereas promastigote forms are seen in sandfly vector.
**Life Cycle**
The life cycle of *L. tropica* is similar to that of *L. donovani* except
**Vectors:** The vectors of *L. tropica complex* are *Phlebotomus* sandflies. The following species of sandflies act as vector.
**Mode of transmission:**
€ . The most common mode of infection is through bite of sandflies.
€ . Infection may also sometimes occur by direct contact.
€ . Infection may be transmitted from man-to-man or animal-to-man by direct inoculation of amastigotes.
**Laboratory Diagnosis**
*Microscopy*

- Smear is made from the material obtained from the indurated edge of nodule or sore and stained by Giemsa or Leishman stain.
- Amastigotes are found in large numbers inside the macrophages.
- Definitive diagnosis is made by demonstration of amastigote in the smear collected from the lesion.

**Pathology**: ulceration necrosis. **Papule** and **ulcer** are the main pathological lesions.

**Laboratory Diagnosis**
*Microscopy*

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