

- بِسْمِ اللّٰهِ الرَّحْمٰنِ  
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- **Visceral  
leishmaniasis**

- Objectives
- Discuss
  - Epidemiology , etiology, lifecycle, transmission
  - Pathogenesis
  - Clinical features
  - Lab diagnosis
  - and treatment
- of visceral leishmaniasis

- *Leishmania* species
- Kingdom protozoa
- Phylum sarcomastigophora
- Subphylum mastigophora (the flagellates)
- Hemoflagellate
- Leishmania classification
- Infection in humans is caused by ~20 *Leishmania* species (*Leishmania* and *Viannia* subgenera)
- Infection caused by leishmaniasis is called leishmaniasis
- Clinical classification
- *Leishmania* species are classified into three clinical groups based on site of infection

- Leishmania that cause infection on the skin called cutaneous leishmaniasis
  - *L. tropica*
  - *L. major*
  - *L. aethiopica*
  - *L. mexicana*
- Leishmania species that cause infection of both skin and mucous membrane (mucous membranes of the nose, mouth and throat cavities)
  - *L. braziliensis*
- Leishmania that causes infection of the deep viscera
  - *L. donovani*
  - *L. infantum*
- Geographic classification
- Old world leishmaniasis is caused by
  - *L. tropica*
  - *L. major*
  - *L. aethiopica*
  - *L. donovani*

- New world leishmaniasis is caused by
  - *L. braziliensis*
  - *L. mexicana*
- History
- The parasite was named in 1903 after the Scottish pathologist William Boog Leishman who observed oval bodies in 1901, while examining pathologic specimens of a spleen from a patient who had died of visceral leishmaniasis.
- Epidemiology
- *Leishmania* currently affects 12 million people in 98 countries.

There are ~ 2 million new cases each year

- Transmission
- Transmitted to humans by the bite of ~30 species of sandflies [*Phlebotomus* (Old World) and *Lutzomyia* (New World)]
- The sand fly injects the infective form 'promastigote' in humans
- Morphology
- *Leishmania* exist in two forms:
  - the Amastigote,
    - the intracellular form (cells of reticuloendothelial system) in the vertebrate host. The amastigote, literally means "without a flagellum," (although not totally devoid of it) It is rounded, non-motile form and divides by binary fission. The amastigote is also called the Leishman-Donovan (LD) body.
  - the Promastigote

- The extracellular form in the sandfly. The promastigote, literally the body form with “an anterior flagellum” ; it is motile, and grows by longitudinal binary  
Promastigotes can be grown in culture.
- Life cycle
- Pathogenesis
- The pathogenesis involves intracellular survival within the macrophage (safe from the immune response) and formation of a granulomatous reaction
- Macrophages containing parasite proliferate in reticuloendothelial organs (liver, spleen, bonemarrow and lymphnodes) resulting in their enlargement
- Proliferation of parasite containing macrophages in bonemarrow kill normal hematopoitic cells

- Visceral leishmaniasis
- Leishmania donovani
- also known as **kala-azar, black fever, and Dumdum fever**
- The clinical features include
  - Prolonged fever; weight loss
  - Parasitic invasion of spleen and liver results in Hepatosplenomegaly (with spleen sometimes massively enlarged);
  - Lymph nodes enlargement
  - Parasitic invasion of bone marrow results in encroachment of normal hematopoietic cells resulting in pancytopenia ;
    - Anemia (fatigue);
    - leukopenia (increased risk of infections);
    - thrombocytopenia (bleeding)
  - Skin blackening
- Post kala-azar dermal leishmaniasis

- Some time after successful treatment—a secondary form of the disease may set in, called post kala-azar dermal leishmaniasis, or PKDL. This condition manifests first as small, measles-like skin lesions on the face, which gradually increase in size and spread over the body

- Lab diagnosis
- Microscopy
- Culture
- Animal inoculation
- Serology
- PCR
- Skin test
  
- Specimens



- Visceral leishmaniasis
  - Peripheral blood
  - Bone marrow aspirate
  - Spleen aspirate
- Microscopy
- Smears(peripheral blood, bone marrow aspirate, spleen aspirate) are stained by Leishman or Giemsa stain and examined under the oil immersion lens. Amastigote forms(LT/LD bodies) can be seen within macrophages and outside
- Culture
- Novy-McNeal-Nicolle(NNN) medium
  - This is a blood agar slope with overlay of Locke's solution (normal

saline+filtered urine) with added antibiotics in screw capped bottles.

- Incubated at 24 °C for 7 days
- Promastigote (in clusters) forms grow and can be demonstrated by examining a drop of fluid under microscope after staining
- Animal inoculation
- The clinical specimen material is inoculated in hamsters intraperitoneally and intradermally. The animals are kept at 25 °C. the parasite is demonstrated in smears from spleen.
- Serology
- Specific
  - Antibody detection by ELISA
  - immunochromatographic dipstick testing of fingerstick blood for

antibody to rK39 antigens (visceral leishmaniasis)

- Nonspecific
  - NAPIER'S ALDEHYDE TEST: 1ml of clear serum of patient + drop of formalin → shake and kept at room temp → Jellification & opacification within 3 – 30min (POSITIVE)
  - CHOPRA'S ANTIMONY TEST: 0.2ml serum diluted 1 in 10ml distilled water →, Overlaid by 4% urea stibamine → thick flocculent disc within 10 – 15min (POSITIVE)
- Skin test
- MONTENEGRO SKIN TEST---- 0.1ml of killed promastigote antigen intradermally read after 72hrs.
- POSITIVE: dermal leishmania & recovered from kala azar.  
NEGATIVE: active case of kala azar

- PCR
- DNA amplification by PCR
- Treatment
- **Any of the following regimens**
- **Visceral Leishmaniasis**
  - Parenteral therapy
    - Pentavalent antimony IV or IM 28 days
    - Liposomal Amphotericin B
    - Paromomycin for ~21 days
    - Pentamidine IV, IM thrice weekly for ~15–30 doses
  - Oral therapy
    - Miltefosine for 28 days
- Prevention
- Sand fly control