B- The blood and tissue flagellates

- Members of the clinically significant group of parasites located in blood and tissue that move by means of flagella, known as the hemoflagellates, belong to the family trypanosomatidae, include genera *Leishmania* and *Trypanosoma*.

-There are four morphologic forms of clinical significance associated with these hemoflagellates: amastigote, promastigote, epimastigote, and trypomastigote.

- Although the specific life cycle may vary, all the organisms in these two genera involve some combination of the four morphologic forms.

- The transmission of all hemoflagellates is via the bite of an arthropod vector.

- The major difference between the two genera is the primary diagnostic form found in each; for *Leishmania* it is the amastigote and for *Trypanosoma* it is the trypomastigote, with the exception of *Trypanosoma cruzi*, in which amastigotes may also be found.

Genus: *Trypanosoma*

There are two distinct types of human trypanosomes:
(1) Species: *Trypanosoma brucei* , causing African trypanosomiasis, subspecies are:

a. *Trypanosoma brucei gambiense.*
b. Trypanosoma brucei rhodesiense.

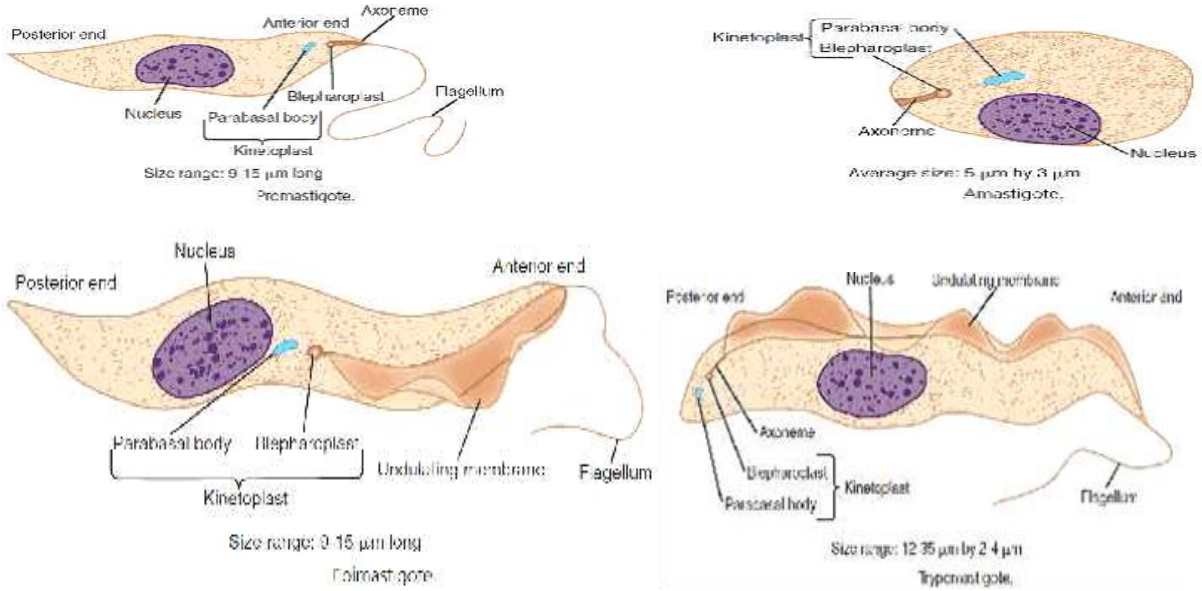
(2) Species: *Trypanosoma cruzi*, causing American trypanosomiasis.

General Characteristics

- They live in the blood and tissues of man and other vertebrate hosts and in the gut of the insect vectors.
- Members of this family have a single nucleus, a kinetoplast, and a single flagellum.
- Nucleus is round or oval and is situated in the central part of the body.

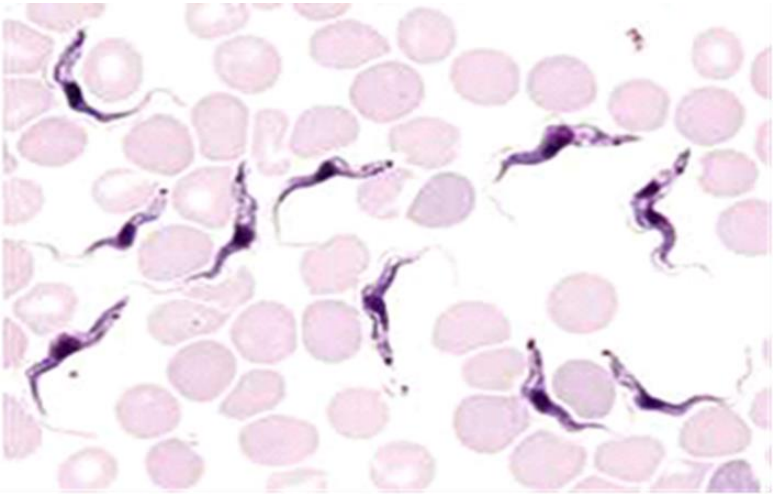
- Kinetoplast consists of a deeply staining parabasal body and adjacent dot like blepharoplast. The parabasal body and blepharoplast are connected by one or more thin fibrils.
- Flagellum is a thin, hair like structure, which originates from the blepharoplast. The portion of the flagellum, which is inside the body of the parasite and extends from the blepharoplast to surface of the body is known as axoneme.
- Hemoflagellates exist in two or more of four morphological stages. These forms were amastigote, promastigote, epimastigote and trypomastigote. The names of the stages are referring to the arrangement of the flagella in relation to the position of the nucleus and its point of emergence from the cells.
- Staining characteristics of trypanosomes: For smears of body fluids, Wrights stain, Giemsa stain, and Leishman's stain are suitable for identifying internal structures.
- All members of the family have similar life cycles. They all require an insect vector as an intermediate host.
- Multiplication in both the vertebrate and invertebrate host is by binary fission. No sexual cycle is known.

Morphology



Trypanosoma brucei gambiense

The genus Trypanosoma appears in the blood as trypomastigotes, with elongated bodies supporting a longitudinal lateral undulating membrane and a flagellum that borders the free edge of the membrane and emerges at the anterior end as a whiplike extension. *T.b. gambiense* (West African Trypanosomiasis), (which causes a sleeping sickness).

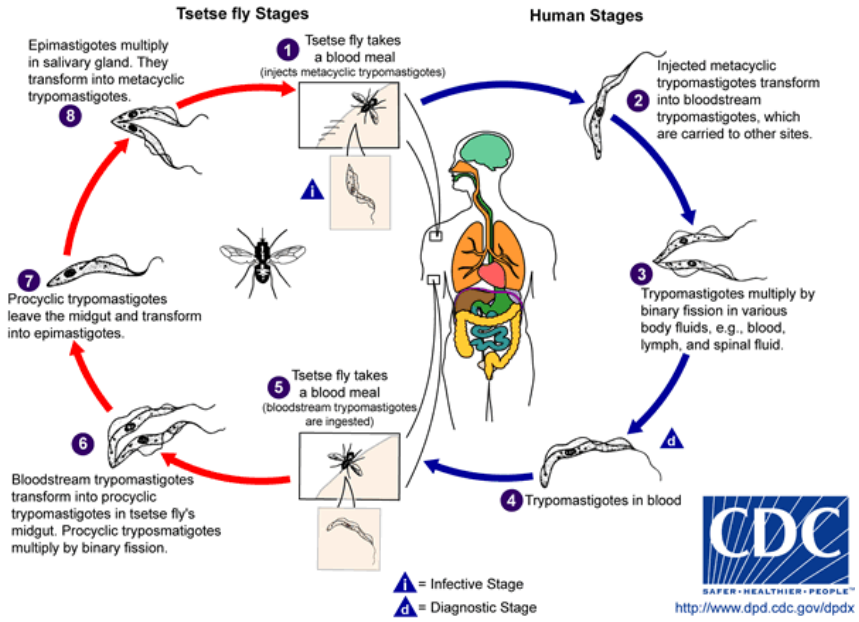


Epidemiology

African trypanosomiasis is restricted to recognized tsetse fly. *T.b. gambiense,* transmitted by the tsetse *Glossina palpalis*, extends from West to Central Africa and produces a relatively chronic infection with progressive CNS involvement.

Life Cycle

T.b. gambiense passes its life cycle in two hosts:
Vertebrate host: Man and animals.
- Invertebrate host: Tsetse fly (In insects, it occurs in 2 forms: epimastigotes and metacyclic trypomastigote forms).
- Both male and female tsetse fly of Glossina species (*G. palpalis*) are capable of transmitting the disease to humans.
-Infective form: Metacyclic trypomastigote forms are infective to humans.
- Mode of transmission: by bite of tsetse fly and congenital transmission has also been recorded.
- Reservoirs: Man is the only reservoir host, although pigs and others domestic animals can act as chronic asymptomatic carriers of the parasite.



1- During a blood meal on the mammalian host, an infected tsetse fly (genus Glossina) injects metacyclic trypomastigotes into skin tissue. The parasites enter the lymphatic system and pass into the bloodstream .
2- Inside the host, they transform into bloodstream trypomastigotes,
3- Trypomastigotes carried to other sites throughout the body, reach other blood fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission
4- The entire life cycle of African Trypanosomes is represented by extracellular stages.
5- The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host
6- In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission.
7- leaves the midgut, and transform into epimastigotes .
8- The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission.

The cycle in the fly takes approximately 3 weeks. Humans are the main reservoir for *Trypanosoma brucei gambiense*, but this species can also be found in animals. Wild game animals are the main reservoir of *T. b. rhodesiense*.

Pathology and Pathogenesis

Human African trypanosomiasis is infection with protozoa of the species *Trypanosoma brucei*, transmitted by the bite of a tsetse fly. Symptoms include characteristic skin lesions, intermittent fever, headache, rigors, transient edema, generalized lymphadenopathy, and often fatal meningoencephalitis.

Infective trypanosomes of *T.b. gambiense* is introduced through the bite of the tsetse fly and multiply at the site of inoculation to cause variable induration and swelling (the primary lesion), which may progress to form a trypanosomal ulcer The African forms multiply extracellularly as trypomastigotes in the blood as well as in lymphoid tissues. They spread to lymph nodes, to the bloodstream, and, in terminal stages, to the central nervous system (CNS), where they produce the typical sleeping sickness syndrome: fatigue, inability to eat, tissue wasting, unconsciousness, and death.

CNS involvement is most characteristic of African trypanosomiasis.*T. b.gambiense* appears in the cerebrospinal fluid in in several months. *T.b. gambiense* infection is chronic and leads to progressive diffuse meningoencephalitis, with death from the sleeping syndrome usually following in 1–2 years. The trypanosomes are transmissible through the placenta, and congenital infections occur in hyperendemic areas.

**Rhodesian brucei* trypanosomiasis becomes symptomatic 1 to 3 weeks after infection, develops rapidly, is more disabling, and often causes death within 3 to 6 months in an untreated patient. Gambian trypanosomiasis causes chronic infection with a more prolonged course.

Trypanosoma cruzi

Trypanosoma cruzi is the causative organism of Chagas's disease or South American
trypanosomiasis. It is a zoonotic disease and is limited to South and Central America.

Trypanosoma cruzi has three developmental stages: epimastigotes in the vector, trypomastigotes (in the bloodstream), and a rounded intracellular stage, the amastigote.

The blood forms of *T cruzi* are present during the early acute stage and at intervals thereafter in smaller numbers. They are typical trypomastigotes with a large, rounded terminal kinetoplast, but they are difficult to morphologically distinguish from African trypanosomes. The tissue forms, which are most common in heart muscle, liver, and brain, develop as amastigotes that multiply to form an intracellular colony after invasion of the host cell or phagocytosis of the parasite.

Life Cycle

- *T. cruzi* passes its life cycle in 2 hosts: definitive host (human) and intermediate host (vector) (Reduviid bug or triatomine bugs).
- Reservoir host: cat, dog, and pigs.

An infected triatomine insect vector (or "kissing bug") takes a blood meal and releases trypomastigotes in its feces near the site of the bite wound.

1- Trypomastigotes enter the host through the wound or through intact mucosal membranes, such as the conjunctiva .

2- Common triatomine vector species for trypanosomiasis belong to the genera Triatoma, Rhodnius, and Panstrongylus. Inside the host, the trypomastigotes invade cells near the site of inoculation, where they differentiate into intracellular amastigotes.

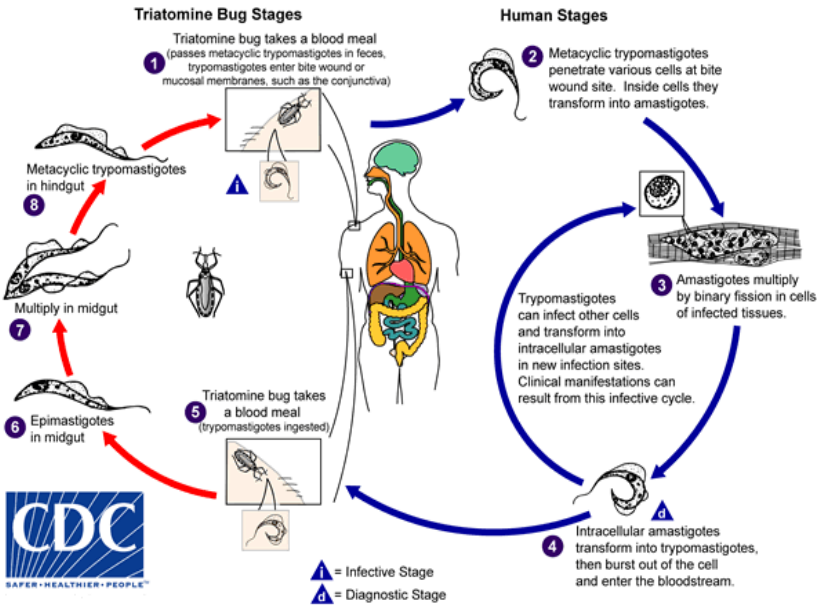
3- The amastigotes multiply by binary fission.

4- Differentiate into trypomastigotes, and then are released into the circulation as bloodstream trypomastigotes.

5- Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle. The bloodstream trypomastigotes do not replicate (different from the African trypanosomes). Replication resumes only when the parasites enter another cell or are ingested by another vector. The "kissing bug" becomes infected by feeding on human or animal blood that contains circulating parasites.

6- The ingested trypomastigotes transform into epimastigotes in the vector's midgut. The parasites multiply and differentiate in the midgut and differentiate into infective metacyclic trypomastigotes in the hindgut.

Trypanosoma cruzi can also be transmitted through blood transfusions, organ transplantation, transplacentally (from mother to unborn baby), and in laboratory accidents.

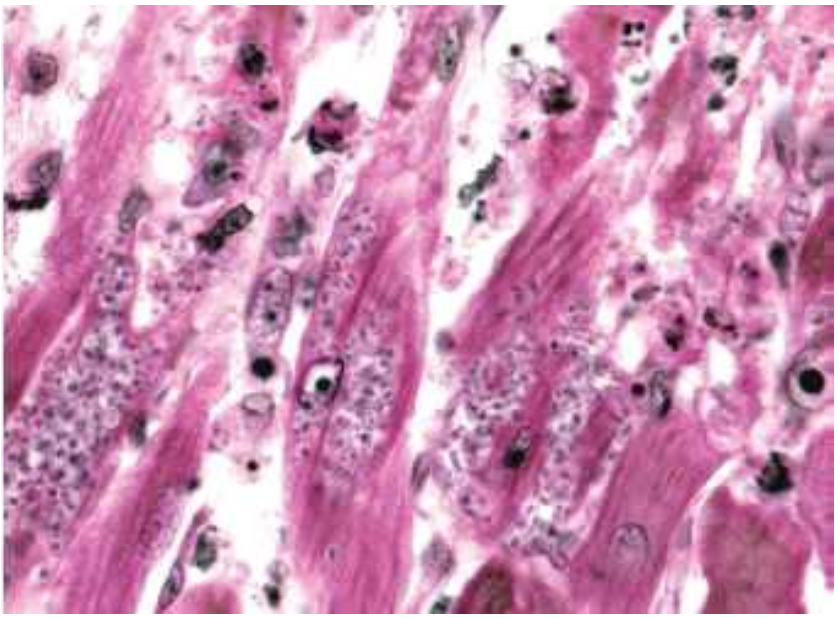


Pathology and Pathogenesis

Infective forms of *T. cruzi* do not pass to humans by bug bites they are introduced when infected bug feces are rubbed into the conjunctiva, the bite site, or a break in the skin. At the site of *T. cruzi* entry, there may be a subcutaneous

inflammatory nodule or chagoma. Unilateral swelling of the eyelids (Romana's sign) is characteristic at onset, especially in children. The primary lesion is accompanied by fever, acute lymphadenitis, and dissemination to blood and tissues.

Interstitial myocarditis is the most common serious condition in Chagas disease. Other organs affected are the liver, spleen, and bone marrow, especially with chronic *T. cruzi* infection. Invasion or toxic destruction of nerve plexuses in the alimentary tract walls leads to mega esophagus and megacolon, especially in Brazilian Chagas disease.



Trypanosoma cruzi amastigote colonies in heart muscle

- ***Leishmania spp.***

The genus *Leishmania*, divided into a number of species infecting humans, causes cutaneous (Oriental sore), mucocutaneous, and visceral (kala-azar) leishmaniasis. All of these infections are transmitted by sandflies (Phlebotomus in the Old World and Lutzomyia in the New World).

Cutaneous leishmaniasis causes painless chronic skin lesions ranging from nodules to large ulcers that can persist for months to years but eventually heal. Mucosal leishmaniasis affects nasopharyngeal tissues and can cause gross mutilation of the nose and palate. Visceral leishmaniasis causes irregular fever, hepatosplenomegaly, pancytopenia, and polyclonal hypergammaglobulinemia with high mortality in untreated patients.

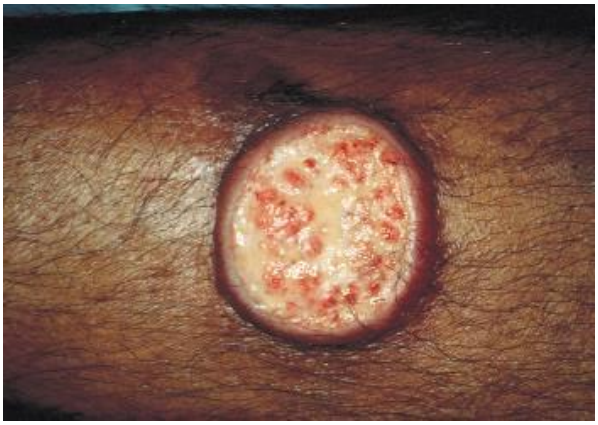
Cutaneous leishmaniasis is also known as oriental or tropical sore, Delhi or Aleppo boil, The causative agents are:

- *L. tropica* Middle and Far-East, Mediterranean area.
- *L. major* Central Asia, Middle-East and Africa

They cause old world cutaneous leishmaniasis,

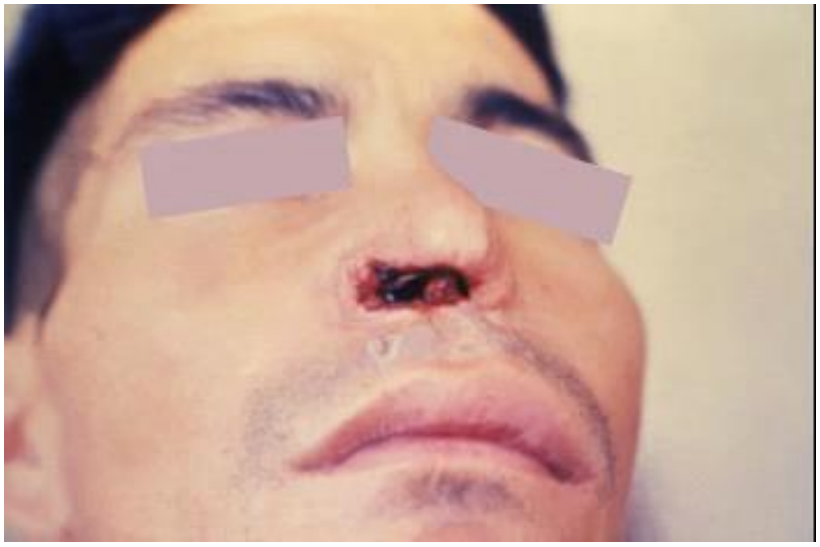
- *Leishmania mexicana* and *Leishmania braziliensis*, they cause new world cutaneous and
  mucocutaneous leishmaniasis, respectively, in Central and South America.

Cases have occurred among US military personnel serving in Iraq and Afghanistan and among travelers to endemic areas in Central and South America, Israel, and elsewhere. Uncommonly, *L. braziliensis* spreads widely in the skin causing disseminated cutaneous leishmaniasis.



Cutaneous leishmaniasis

1- **Mucosal leishmaniasis** :is caused mainly by *L. braziliensis* but occasionally by other *Leishmania sp.* The parasites are thought to spread from the initial skin lesion through the lymphatics and blood to nasopharyngeal tissues. Symptoms and signs of mucosal leishmaniasis typically develop months to years after the appearance of the skin lesion.



Pathogenicity: Cutaneous leishmaniasis is characterized by:

1. Multiplication of amastigotes in the skin macrophages leading to formation of papule, nodule and ulcer.
2. The ulcer may be single or multiple, that heals over months to years, leaving scar.
3. Recovery from cutaneous leishmaniasis gives a life-long immunity against the same *Leishmania* species.

Diagnosis:

- Clinical diagnosis: The type of lesion is a helpful feature.
1. Microscopy: For detection of amastigotes in:
- Smears aspirated or scraped from the edge of the lesion and stained with Leishman, Giemsa or Wrights stain.
- Biopsy of skin lesion stained with H & E.
2. Culture: Materials are cultured on NNN media to see promastigotes.
3. Animal inoculation.
4-Leishmanin skin test (Montenegro test)
5. Serological tests.

Treatment:
1. Local measures:
- Surgical excision especially in single lesions.
- Scraping .
- Local injection of 10% atebrine solution.
2. Systemic treatment:
Pentostam is the drug of choice and (oral) Miltefosine.

2- **Visceral leishmaniasis** (kala-azar, Dumdum fever) is typically caused by *L. donovani* or *L. infantum*(previously called *L. chagasi* in Latin America) and occurs in India, Africa (particularly the Sudan), Central Asia, South and Central America, and infrequently China. Most cases occur in northeastern India. Parasites disseminate from the site of the sand fly bite in the skin to regional lymph nodes, the spleen, the liver, and bone marrow and cause symptoms. Subclinical infections are common; only a minority of infected patients develop progressive visceral disease. Symptomatic infection with *L. infantum* is more common among children than adults. Visceral leishmaniasis is an opportunistic infection in patients with AIDS or other immunocompromising conditions.

Morphology:

1. Amastigote form (Leishman Donovan body): In reticulo endothelial cells (RECs) all over the human body and reservoir host (vertebrate hosts), typically intracellular in macrophages.

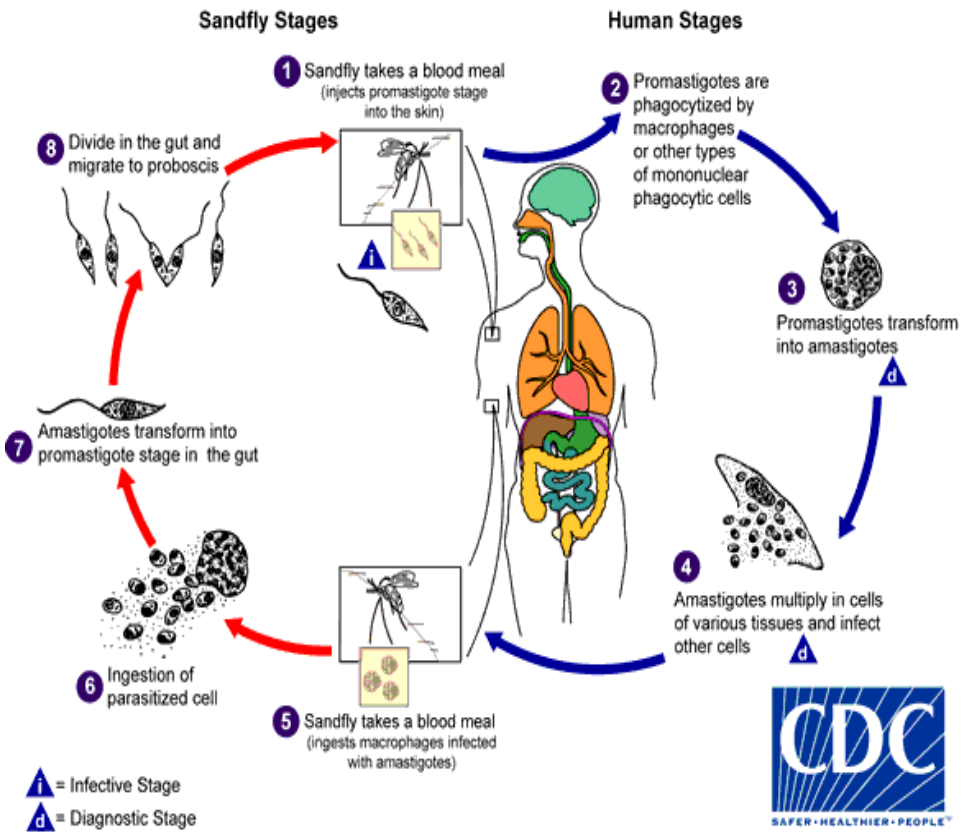
2. Promastigote form: In insect vector (invertebrate host) and culture.

Life cycle:

- Habitat: RECs of viscera, especially spleen, liver, bone marrow, intestinal mucosa and mesenteric lymph nodes.
- Definitive host: Man.
- Reservoir host: Dogs, rodents, wild and domestic animals.
- Insect vector: Female sand flies of the genus Phelebotomus in the old world, and Lutzomyia in the new world.
- Infective stage: Promastigotes.

* Man acquires the infection when the infected female sand fly attempts a bloodmeal, where some of the promastigotes in the buccal cavity are regurgitated, and introduced into the skin bite by their motility.

- Promastigotes are phagocytosed by skin macrophages, where they metamorphose into amastigotes that reproduce by binary fission.
- Ruptured parasitized cells release large number of amastigotes into circulation.
- Blood monocytes phagocytose the free amastigotes and carry them to the viscera, where they produce generalized infection of the RECs.



Pathogenesis

In visceral leishmaniasis, the organs of the reticuloendothelial system (liver, spleen and bone marrow) are the most severely affected organs. Reduced bone marrow activity, coupled with cellular distraction in the spleen, results in anaemia, leukopenia and thrombocytopenia. This leads to secondary infections and a tendency to bleed. The spleen and liver become markedly enlarged, and hypersplenism contributes to the development of anaemia and lymphadenopathy also occurs. Increased production of globulin results in hyperglobulinemia, and reversal of the albumin-to-globulin ratio.

Diagnosis

- Light microscopy of tissue samples, touch preparations, or aspirates; when available, PCR-based assays
- For visceral leishmaniasis, antibody titers
- For cutaneous and mucosal leishmaniasis, skin testing (not available in the US)
- Culture (special media required)
- PCR-based assays of aspirates from bone marrow, the spleen, or lymph nodes in patients with visceral leishmaniasis or of biopsy.

Treatment

liposomal amphotericin IV, amphotericin B deoxycholate IV, or miltefosine.

Prevention

For prevention, the following may help:

- Treatment of leishmaniasis in a geographic area where humans are a reservoir.
- Reduction of the vector population by spraying residual insecticide (one that has prolonged duration of action) in sites of domestic transmission
- Control of nonhuman reservoirs.