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Heamoflagellates

Haemoflagellates of medical importance belong to family Trypanosomatidea and have two genera :

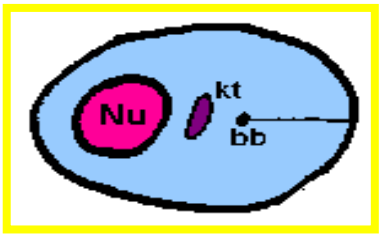
- ***Trypanosoma***
- ***Leishmania***

Common features of these parasites are:

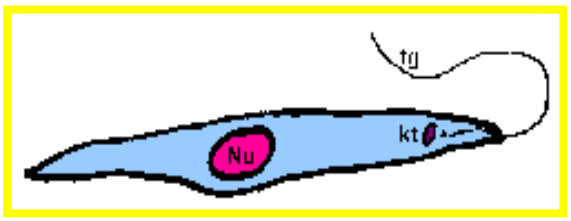
1. All members of the family have similar life cycles. They all require an insect vector as an intermediate host.
2. Parasites metamorphose during development but the number of development stages and tissue tropism are different for each species.
3. They live in the blood and tissues of man and other vertebrate host, and in the gut of the insect vector.
4. Multiplication in both the vertebrate and invertebrate host is by binary fission. No sexual cycle is known.
5. Haemoflagellates exist in two or more of four morphological stages.
6. Heamoflagellate has a nucleus, kinetoplast and a single flagellum. The kinetoplast consists of a deeply staining parabasal body and the adjacent dot-like blepharoblast. The blepharoplast and parabasal body are connected by one or more delicate fibrils. The flagellum arise from the blepharoplast. The portion of the flagellum which is inside the body of the parasite is called as axonemes or axial filament.

Morphological stages of heamoflagellates

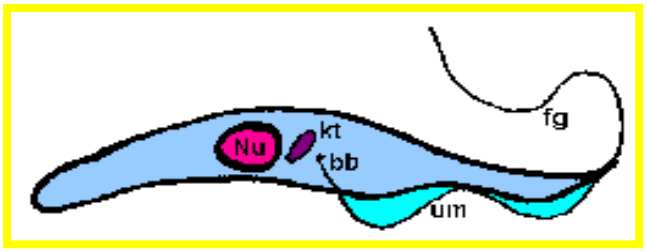
- **Amastigotes stage**: The roundish to oval amastigote measures 5 by 3 Mm in size. The amastigote consists of a nucleus and a kinetoplast. The large single nucleus is typically located off-center, some time present more toward the edge of the organism. The dote like blepharoplast gives rise to and is attached to a small axonemes. The axonemes extends to the edge of the organism. The single parabasal body is located adjacent to the blepharoplast.



- **Promastigotes**: measure 9 to 15Mm in length. The large single nucleus is located in or near the center of the long and slender body. The kinetoplast is located in the anterior end of the organism. A single free flagellum extends anteriorly from the axonemes.

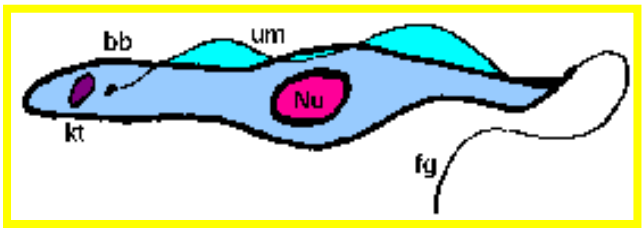


- **Epimastigotes:** measures approximately 9 to 15Mm in length. The body is slightly wider than that of the promastigote. The large single nucleus is located in the posterior end of the organism. The kinetoplast is located anterior to the nucleus. An undulating membrane measuring half the body length forms into a free flagellum at the anterior end of the epimastigote.



- **Trypomastigotes**: measures 12-35Mm long by 2-4 Mm wide, and may often assume the shape of the letters C or U in stained blood film. The long, slender organism is characterized by a posteriorly located kinetoploast from which emerges a full body length undulating membrane. The single, large

nucleus is located anterior to the kinetoplast. An anterior free flagellum may or may not be present.



Genus :*Leishmania*

Species of *Leishmania* are digenetic (heteroxenous)parasitic protozoa of humans and animals that are found alternatively as flagellated, motile promastigotes in the alimentary tract of phlebotomine sandflies, or as obligate intracellular aflagellate amastigotes in the phagolysosomes of mammalian host macrophages. There are over 25 designated species plus several unnamed species grouped into two subgenera.

Leishmaniasis presents in four different forms with a broad range of manifestations:

- **Visceral leishmaniasis**:(VL, kala azar) is the most severe form and if untreated has a mortality rate approaching 100%. This form is

characterized by fever, weight loss, enlargement of the spleen and liver and anaemia. Caused exclusively by species of the *L. donovani* complex (***L. donovani, L. infantum* , *L. chagasi*)**

- **Mucocutaneous leishmaniasis**:(MCL) produces lesions which can lead to extensive and disfiguring destruction of mucous membranes

of the nose, mouth and throat cavities.

- **Cutaneous leishmaniasis** (CL) manifests with sores or ulcers on

exposed parts of the body such as arms, legs and face which may heal spontaneously, but the diffuse form of CL does not heal and may relapse after treatment.

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Species of Leishmania parasitic to man

Leishmania tropica: causing oriental sore, the infection is limited to local lesions of the skin and subcutaneous tissues.
Leishmania donavani: causing kala-azar, the infection is generalized and the parasites are distributed in the internal organs, therefore the disease is called visceral leishmaniasis.
Leishmania braziliensis: causing Espundia, the infection is limited to local lesion of the skin and nasopharyngeal mucous membrane.
Leishmania infantum: is found along the whole Mediterranean area , human infections are confined almost entirely to children, humans are considered to be an accidental host, with natural infections occurring in dogs.

Visceral leishmaniasis

Morphology and life cycle

The sand fly vector becomes infected when feeding on the blood of an infected individual or an animal reservoir host. The *leishmania* parasites live in the macrophages as round, non-motile amastigotes (3-7 micrometers in diameter). The macrophages are ingested by the fly during the blood- meal and the amastigotes are released into the stomach of insect. Almost immediately the amastigotes transform in to the motile, elongated (10-20 micrometers), flagellate promastigote form. The promastigotes then migrate to the alimentary tract of the fly, where they live extracellularly and multiply by binary fission. Four to five days after feeding the promastigotes move forward to the oesophagus and the salivary glands of the insect. When the sandlfy next feeds on a mammalian host, it's proboscis pierces the skin and saliva containing anti-coagulant is injected into the wound to prevent the blood from clotting, the *leishmania* promastigotes are transferred to the host along with the saliva. Once in the host the promastigotes are taken up by the macrophages where they rapidly revert to the amastigote form. The *leishmania* are able to resist the microbiocidal action of the acid hydrolases release form the lysozymes and so survive and multiply inside the macrophages, eventually leading to the lysis of the macrophages. The released amastigotes are taken up by additional macrophages and so the cycle continues. Ultimately all the organs containing macrophages and phagocytes are infected, especially the spleen, liver and bone marrow and less often in other locations such as the skin, intestinal mucosa and mesenteric lymph nodes. Small number of LD bodies can be found in peripheral blood inside polymorphnuclear leukocytes or monocytes. Rarely may be seen in feces, urine and nasal secretions.

Insect vectors

The only proven vector of the *leishmania* parasite is the blood-sucking female of the genus *Phlebotomus* in the old world and *Lutzomyia* in the new world. The insects are 2-3 mm long and are found through-out the tropical and temperate parts of the world. The sandfly larvae require organic matter, heat and humidity for development and so are commonly found in house-hold rubbish, bark of old trees, burrows of old trees and in cracks in house walls. The sandflies usually feed at night while the host is asleep.

Clinical feature

Various terms have been used to describe visceral leishmaniasis including Dum-dum fever, Sikari disease, Burdwan fever, Shahib's disease and tropical splenomegaly. The most commonly used term is Kala azar, which means black sickness or black fever. The terms originally referred to Indian VL due to its characteristic symptoms, blackening or darkening of the skin of the hands, feet, face and the abdomen .

Visceral leishmaniasis is caused by the parasites *Leishmania donovani donovani*, *Leishmania donovani infantum* in the old world and by *Leishmania donovani chagasi* in the new world. In endemic cases of VL, the disease is chronic and onset is gradual. Although people of all ages are susceptible in the old world, children below the age of 15 are more commonly affected with *L.d infantum* being largely responsible . In sporadic and epidemic cases of VL the disease is usually acute and symptoms appear suddenly with people of all ages being at risk except those who have conferred immunity due to a past infection.

The symptoms of VL vary between individuals and according to geographical foci. some of the common symptoms include high undulating fever often with two or even three peaks in 24 hours and drenching sweats which can easily be misdiagnosed as malaria, Chills, rigors, weight loss, fatigue, poor appetite, cough, burning feet, insomnia, abdominal pain, joint pain, anorexia, epistaxis and diarrhoea. Clinical sings include splenomegaly, hepatomegaly and lymphadenopathy . The incubation period is highly variable, the disease can appear anything between ten days to over one year. Also the skin becomes dry, rough and darkly pigmented, the hair becomes thin and brittle. Epistaxis and bleeding gums are common.

Visceral leishmaniasis can be complicated by serious secondary bacterial infections such as pneumonia, dysentery and pulmonary tuberculosis, which often contribute to the high fatality rate of VL patients. Other complications, though rare include haemolytic anemia, acute renal damage and sever mucosal haemorrhage .

Post kala-azar dermal leishmaniasis (PKDL) is a recurrence of Kala-azar that may appear on the skin of affected individuals up to 20 years after being partially treated, untreated or even in those considered adequately treated.. They manifest as hypo-pigmented macules, papules, nodules, or facial erythema (butterfly patches). Though any organism causing Kala-azar can lead to PKDL, it is commonly associated with *L. donovani* which gives different disease patterns in each country.

Pathology

In speen: macroscopic: Grossly enlarged, capsule thickened, soft in consistency, marked congestion.

Microscopic: Vascular spaces widely dilated and engorged with blood. Reticular cells markedly increased and paked with L-D bodies. No fibrosis and plasma cell increased.

In liver: Macroscopic: liver is enlarged and congested

Microscopic: Kupffer cell hyperplasia and these cells are loaded with L-D bodies. Sinusoidal capillaries are dilated and engorged with blood.

Bone marrow: Haematogenous tissue replaced by proliferated and parasitized macrophage and increased number of plasma cells. LD bodies are in plenty.

Diagnosis

Clinical

Preliminary diagnosis is based on the symptoms and clinical signs of visceral leishmaniasis such as splenomagaly, hepatomegally and high undulating fever. However these alone are not enough to differentiate VL from other similar conditions such as malaria, relapsing fever, liver abscess and trypanosomiasis.

Parasitological

Spleen and liver biopsy

Looking for parasites in the spleen and liver is one of the most accurate methods available to determine *leishmanial* infections. The smallest needle possible, preferably, 21-gauge (0.8 mm) should be used to minimise the risk of complications such as haemorrhage of the spleen . Part of the splenic aspirate can be used to make smears for direst microscopic examination and the rest should be cultured. *L. donovani* grows well on Novy- MacNeal- Nicolle (NNN) or Schneider's insect medium supplemented 10% v/v foetal calf serum, although other suitable growth media can be used just as well. Liver biopsy material is less likely to demonstrate parasites on direct examination or on culture, however histological examination will show amasatigotes in Kupffer cells in the portal system.

Marrow and lymph gland puncture

Marrow obtained from sternal or iliac crest puncture is a much safer but a painful method. It is less likely to demonstrate parasites in direct stained films, however, on culture it can give positive results in up to 80% of the cases. Lymph gland puncture gives positive results in 60% of the cases. Juice is extracted from any enlarged lymph gland and subjected to both direct examination and culture to give the best chance of diagnosis.

Blood buffy coat

Finding the *leishmania* parasites in blood in sometimes possible in patients with Kala-azar . Blood in anticoagulant is centrifuged at 2000g for 10 min and the cells from the buffy coat removed and used to prepare smears and inoculate cultures. Amastigotes can be found in and around Macrophage cells. The volume used in culture inoculation is important, 1-3 drops on NNN or Schneider's medium has given successful results

Serological

Serological diagnosis is based on the presence of specific humarol antibodies in cases of visceral leishmaniasis. This includes:

- **Indirect fluorescent antibody test (IFAT)**
- **Direct agglutination test**
- **Enzyme linked immunosorbent assay (ELISA)**
- **Complement fixation test**

- **PCR technique, this method based on the amplification of the leishmania DNA by using specific primer which depend on the sequence of nitrogen bases in the leishmania genome.**

Treatment

Pentavalent antimonial are the drug of choice for treatment.
Aminosidine ointment has been found to be useful as local treatment.

Cutaneous leishmaniasis

Oriental sore, as seen in the Old World, is produced by Leishmanias belonging to the Leishmania tropica complex. There are serologicaly and biochemically distinct species, all transmitted by sandflies belonging to the genus Phlebotomus.
L. tropica produces chronic disease that, if not treated, lasts for a year or longer.
L. tropica minor: cause urban cutaneous leishmaniasis, dry type **,** reservoir host is dog.
L. tropica major: cause rural cutaneous leishmaniasis, wet type, reservoir host is rodent, produces an acute infection with a duration of 3 to 6 months. The lesions occur primarily on the lower limbs, they are moist and tend to ulcerate very early; there may be secondary or satellite lesions.
L. aethiopica produce a similar chronic disease,seen in the highland of Ethiopia

Morphpogy and life cycle

The appearance of the the amastigotes of L. tropica and L. major is similar to that of the other leishmanias of human. Sand flies of the genus Phlebotomus are the intermediate hosts and vector. When the fly takes a blood meal containing amastigotes, the parasite multiply in the midgut and then move to the pharynx, they are then inoculated into the next mammalian victim. There they multiply in the reticuloendothelial system and lymphoid cells of the skin.

Pathogenesis

The incubation period lasts from a few days to several months. The first symptom of infection is a small, red papule at the site of the bite, which may itch intensely and grows to 2 cm or more in diameter. In L. major infections, the papule is covered with a serous exudates and ulcerates early; papules are dry and ulcerate only after several months in L. tropica and L. aethiopica infections.. This papule

may disappear in few weeks, but usually it develops a thin crust that hides a spreading ulcer underneath. Two or more ulcers may coalesce to form a large sore . In uncomplicated cases the ulcer will heal within two months to a year, leaving a depressed , unpigmented scar.
L. tropica is found in more densely populated areas. Its lesion is dry, persists for months before ulcerating , and has numeratous amastigotes within it. By contrast, L. major is found in sparsely inhabited regions. Its papule ulcerates quickly, is of short duration, and contain few amastigotes.

Daignosis

Scraping from the side or edge of the ulcer smeared on a slid and stained with Wrights or Giemsa stain will show the parasites in endothelial cells and monocytes, even though they cannot be found in the circulating blood.
Leishmanin or Montenegro test , it is a skin test and is used to measure delayed hypersensitivity. 0.1ml of antigen suspension of washed promastigotes in 0.5 percent phenol saline in a strength of 10 percent is injected intradermally , positive result is indicated by an induration of 5mm or more in 48-72 hours

Treatment

By sodium stibogluconate(less toxic than the earlier pentavalent antimonials,Pentostam) is the most effective compound available for treatment of all cutaneous leishmaniasis except the Ethiopian form of diffuse cutaneous leishnaniasis is more effected by pentavalent antimonials.

Mucocutaneous leishmaniasis

L. brazilinensis produces a disease in humans known as espundia, uta, or mucocutaneous leishmaniasis. Morphologically, *L. braziliensis* cannot be differentiated *from L. tropica, L. mexicana, or L. donovani*.

Life cycle and pathogenesis

The life cycle and methods of reproduction of L. *braziliensis* are identical to those of *L. donovani* and *L. tropica* except that the promastigotes reproduce in the hindgut of the sand fly, with several species of *Lutzomyia* servaing as vectors. Inoculation of promastigotes by of a sand fly causes a small, red papule on the skin. This becomes an itchy, ulcerated vesicle in one to four weeks and is similar at this stage to oriental sore. This primary lesion heals within 6 to 15 months. The parasite never causes a visceral disease but often develops a secondary lesion on some region of the body.

In some times the lesions appear as flat, ulcerated plaques that remain open and oozing. The disease is called **pian bois** in Venezuela and Paraguay.
In more southerly range of *L. braziliensis*, the parasites have a tendency to metastasize , or spread directly from the primary lesion to mucocutaneous zones. The secondary lesion may appear before the primary has healed, or it may be many years before secondary symptoms appear. The secondary lesion often involves the nasal system and buccal mucosa,causing degeneration of the cartilages and soft tissues. Necrosis and secondary bacterial infection are common. **Espudia** and **uta** are the names applied to these conditions. The ulceration may involve the lips, palate, and pharynx, leading to great deformity. Invasion of the infection into the larynx and trachea destroys the voice. The condition may last for many years, and death may result from secondary infection or respiratory complications.

Daignosis and treatment

Diagnosis is established by finding L-D bodies in affected tissues. Espundia like conditions are also caused by tuberculosis, leprosy, syphilis, and various fungal and viral diseases, and these must be differentiated in diagnosis. Treatment is similar to kala-azar and tropical sore, antimonial compounds applied on the lesions or injected intravenously or intramuscularly . Secondary bacterial infections should be treated with antibiotics.

Leishmania mexicana

This parasite of New world is found in northen central America, Mexico,Texas.Three clinical manifestations are found: cutaneous , nasopharyngeal mucosal, and visceral, The cutaneous form of disease has been called **chiclero ulcer** because it is so common in chicleros forest-dwelling people .
Sand flies are the vectors of *L. mexicana*, several species of *Lutzomyia* are involved. The disease is a zoonosis, and the main reservoirs are the rodents.
Cutaneous leishmaniasis due to *L. mexicana* usually heals spontaneously in a few months except when the lesions are in the ear. Ear cartilage is poorly vascularized so immune responses are weak.
Diagnosis and treatment of *L. mexicana* is the same as for *L. tropica*.

Leishmaniasis recidivans: a partially healing leishmanial lesion caused by *Leishmania tropica* and characterized by an extreme form of **cellular immune response**, intense granuloma production, **fibrinoid necrosis** without **caseation**, and frequent development of **satellite lesions** that continue the production of

granulomatous tissue and **scarring** without healing, sometimes over a period of many years; organisms are difficult to demonstrate but can be cultured.

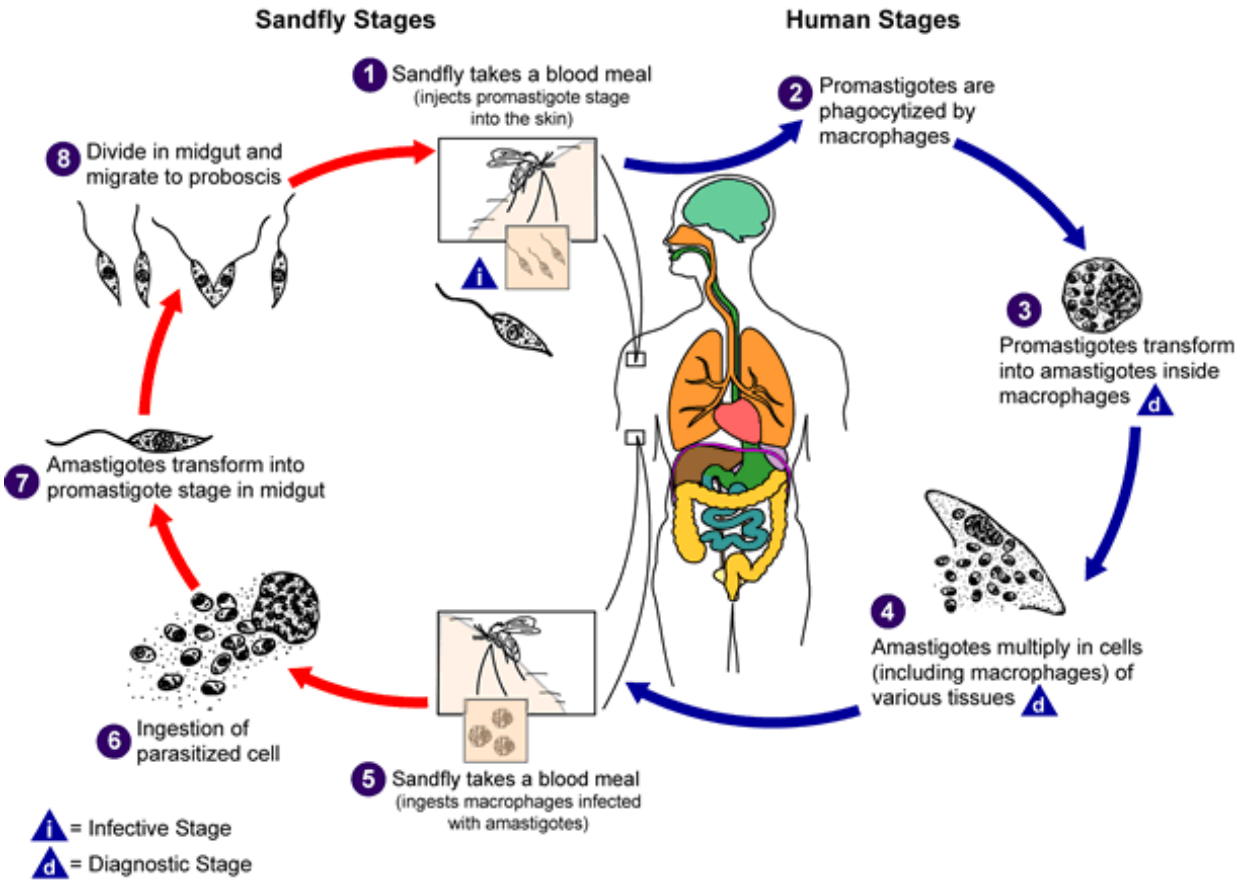
Diffuse cutaneous leishmaniasis: develop in patients lacking adequate cell mediated immunity , characterized by numerous nodular non-ulcerating lesions particularly on the face and limbs, which resemble the lesion of lepromatous leprosy.

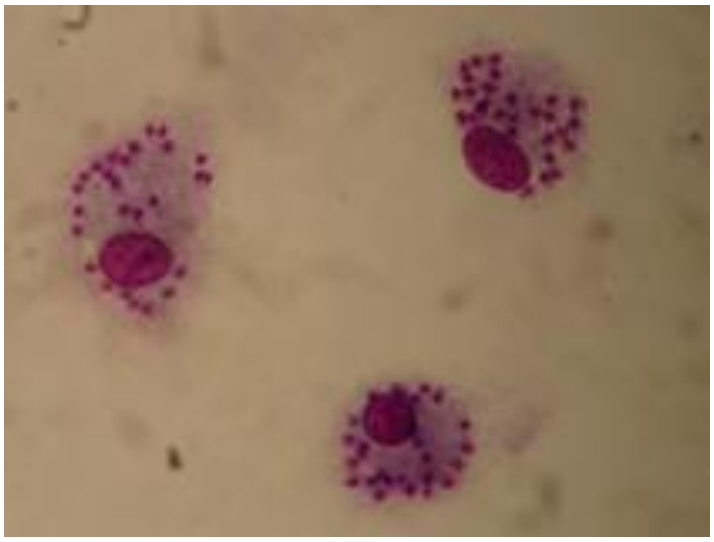
Prevention of Leishmania

So far all attempts to create a preventative vaccine have been unsuccessful. However there is some evidence that people who have had cutaneous infections have heightened resistance to future visceral or cutaneous infections so some researchers are looking into the possibility of infection with an attenuated strain in the epidermis to cause a mild cutaneous infection. At present the only effective preventative measure is to prevent sand fly bites either by killing them with pesticides or by using insect repellents

Epidemiology

The infection produced by L. tropica is generally transmitted from one human to another, the other forms of cutaneous leishmaniasis are principally zoonosis . Although sandflies are the natural vectors of all types of leishmaniasis , contact infection is possible, and vaccination is practiced in certain areas by inoculating serum from naturally acquired lesions into an inconspicuous location on the body of a nonimmune person.Various clinical forms of visceral leishmaniasis are characteristic of different localities. In the urban forms, transmission is primarily from human to human. A rural form of transmission , seen in other areas , is primarly a zoonosis.





Amastigote stage

Promastigote stage