Univ. of Mustansiriya
College of Pharmacy
Branch of Clinical Laboratory Sciences

Medical Parasitology

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Lec. 4 Protozoa

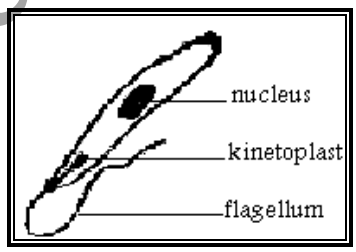
B. Blood and tissue flagellates:

All these organisms require two hosts in their life cycle, man or another susceptible mammal on the one hand and a blood sucking insect on the other hand.

These flagellates related to genus ***Leishmania*** and ***Trypanosoma***. They are passing through many stages in their life cycle which is distributed between the vertebrate host (the final host) and the arthropod host (the intermediate host). These stages are different in their morphology, flagellum position and if it is found or not, the shape of the kinetoplast and its position and the presence or absence of the undulating membrane. They are summarized in the following:

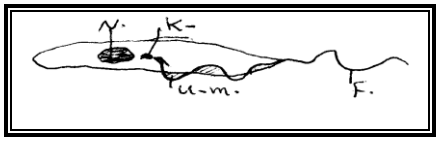
1. Promastigote (leptomonad form)

The body is spindle in shape, the nucleus in the middle of the body (relatively). The kinetoplast nearby the anterior end, the flagellum extends from this structure and continues toward the outside of the body; there is no undulating membrane.



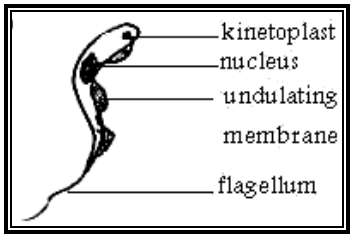
2. Epimastigote (crithidial form)

The body is spindle in shape, the kinetoplast in front of the nucleus which is found nearby the middle of the body, toward the posterior end. There is an undulating membrane, the flagellum grows and extend within the body till the anterior end through the undulating membrane then extends freely.



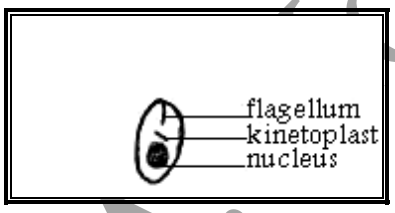
3. Trypomastigote (trypanosomal form)

The body is spindle in shape, the nucleus in the middle of the body, the kinetoplast found in the posterior region of the body; it produces the flagellum which extends on the outer margin of the undulating membrane then extends freely.



4. Amastigote (leishmanial form)

The body is spherical or oval with oval nucleus found nearby the body center; in front of the nucleus, the kinetoplast is laid, which produce a short flagellum extends till the body surface. There is no undulating membrane in this form.



I- *Leishmania*

The vertebrate host is mammals, particularly the human, dogs and many species of rodents. The invertebrate host is sand fly (the vector).

Life cycle:

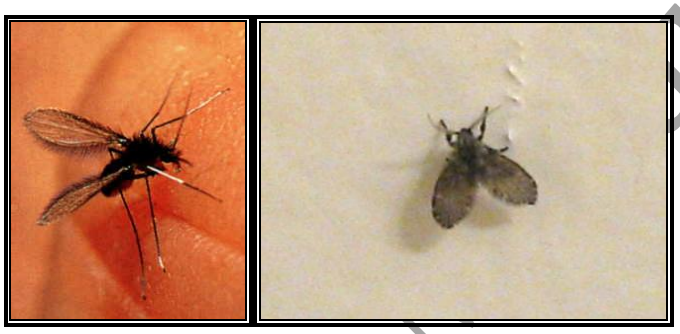
A. The Vertebrate host:

When a sand fly holds the infective stage of Leishmania (the promastigote stage) takes a blood meal from man or other mammals, it will passively inject these parasites which will be picked up later by the reticuloendothelial system and transform to amastigote stage. It is passing through sequence binary fissions until cells are filled with the parasites and burst to release these parasites which may infect other cells. At times, many infected phagocytic cells arrive to circulation and to viscera, where the parasite settles down and multiply in the reticuloendothelial cells of the liver, spleen and boon marrow, causing the destruction of these cells.

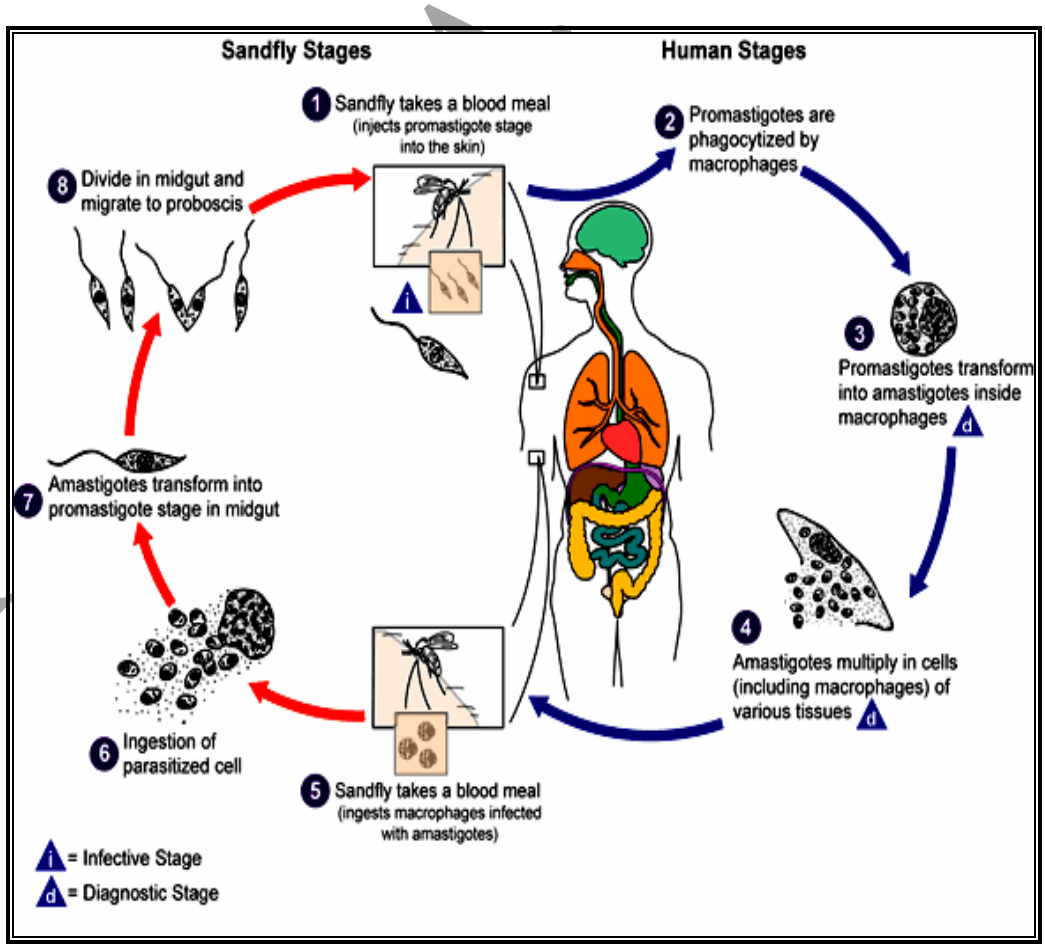
B. The Invertebrate host:

These are many species of **sand fly** from genus ***Phlebotomus***. However, when the insect bites the infected vertebrate host, it will suck the blood and the parasite (amastigote stage) which migrate then to the midgut of the insect and transform to promastigote stage and start in multiplication by binary fission. The parasites may be sticks on the intestinal wall of the insect or stay free in the lumen of the intestine. It may be found in aggregations of promastigote stage in the first and last part of the gut.

After 4 to 5 days from the insect feeding, the promastigotes (the infective stage) will fill the esophagus and when they block the esophagus, the insect will push the esophagus contents to the front and back; by this method it will inject the infective stages in the victim.



Sand fly (the vector of *Leishmania* parasite).



"Diagram for the life cycle of *Leishmania*".

The genus ***Leishmania*** includes the following species:

1. *Leishmania donovani*

This parasite causes a disease called Kala-azar or Dum-Dum fever or visceral Leishmaniasis or black fever. Its vector is the sand fly (***Phlebotomus papatasi***).

In the body of the vertebrate host, the parasite spreads in many parts, particularly, in the endothelial cells of the blood and lymph capillaries of the spleen, liver and bone marrow. Sometimes, the parasite infects the skin again and multiplies there. In this stage, the disease called dermal **Leishmaniasis** or post **Kala-azar**.

Symptoms and Pathology

The incubation period varies from 10 days to many months, but in the usual case, it is insidious. On the average about 90 days following exposure. The symptoms can be observed are:

1. Great enlargement in the spleen and the liver with an increase in the size and number of parasitized Kupffer cells.

2. The bone marrow exhibits markedly increased production of macrophages and decreased erythropoietic function.

3. Thrombocytopenia results in multiple hemorrhages, particularly from the mucous membranes.

In the typical acute case:

1. Temperature fluctuates daily from 37.5 - 40˚C.
2. Bleeding typically occurs from the gum, lips, naris and intestinal mucosa.
3. Complications usually observed in **kala-azar** are principally diarrhea or dysentery and broncho-pneumonia.

Diagnosis

It can be done by using serological methods (serodiagnosis) as well as the demonstration of the parasite itself microscopically in the biopsy specimens taken from the bone marrow, spleen, liver or blood sample. The parasite found inside the macrophages.

Treatment

Pentavalent antimonial drug. The treatment should be given until the aspirate is free of parasites for at least (2) weeks.

2. *Leishmania tropica*

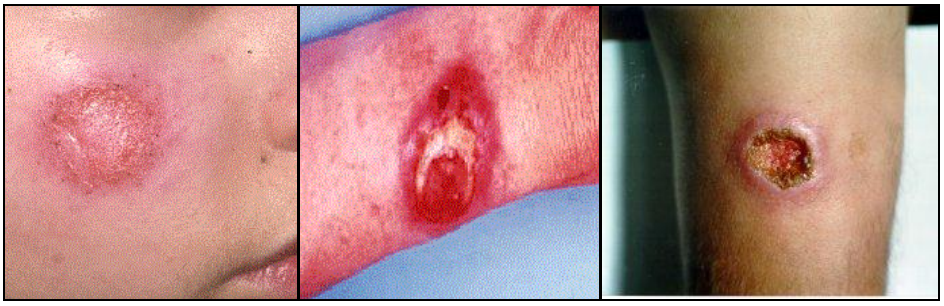
This parasite causes a disease called dry or urban cutaneous Leishmaniasis or oriental sore or Baghdad boil or Old world cutaneous Leishmaniasis or tropica sore. The vector is ***Phlebotomus papatasi*** and ***Phlebotomus sergenti***.

The tissue reaction is initiated with the introduction of the promastigotes into the dermis. The macrophages in the vicinity picks up the parasites which rapidly transform to amastigotes and multiply, destroying the host cells. Soon after, there will be a dense concentration of macrophages in the invaded area, all of which are liable to infection and destruction. The lesion resulted from the insect bite become necrotic at the center, and the margins containing parasitized macrophages which may become infiltrated with giant and plasma cells.

Pathology and Symptoms

The lesion appears in the beginning as a macula, then as a papule, with a slightly raised center covered by a thin blister like layer of epidermis. Then it breaks down, with discharge of a small amount of clear or purulent exudates.

The incubation period many be as short as 2 weeks or as long as 3 years, but usually between 2-6 months. In uncomplicated cases there are no systemic manifestations. The common occurrence of phonic complications causes painful, disfiguring, local ulcer, neutrophilic leukocytosis and fever, and at times septicemia.



Cutaneous lesions in cutaneous leishmaniasis.

Diagnosis

The uncomplicated lesion may be mistaken for a variety of infections of the skin; hence demonstration of the parasite is essential. Examination of Giemsa stained slides of the relevant tissue is still the technique most commonly used to detect the parasite. As well as the serological methods.

Isolation of the organism in culture medium (using for example the diphasic NNN medium) or in experimental animals (hamsters) constitutes another method of parasitological confirmation of the diagnosis. Antibody detection can prove useful in visceral leishmaniasis but it is of limited value in cutaneous disease, where most patients do not develop a significant antibody response.

Other diagnostic techniques exist that allow parasite detection and/or species identification using biochemical (isoenzymes), immunologic (immunoassays) and molecular approaches (PCR). Such techniques, however, are not readily available in general diagnostic laboratories.

Treatment: Pentavalent antimonials.

There is another type of this disease called **muco-cutaneous Leishmaniasis or Espundia** in Brazil or many other tropical parts in America. The infection clinically resembles the oriental sore, but it is caused by ***Leishmania braziliensis.***

The infection starts in the skin but the sores spread to extent regions and appear in a big number on the mucous membranes of the mouth, nasopharynx by extension or metastasis. It is also infecting the ear, nose cartilages and the throat, but not found in the blood and it is seldom in the inner viscera. The life cycle similar to that of ***L. tropica***.

II- *Trypanosoma*

This parasite has two hosts, vertebrate and invertebrate hosts. The invertebrate one (the vector) is **Tsetse fly [*Glossina palpalis*].**

Life cycle:

A. Vertebrate host:

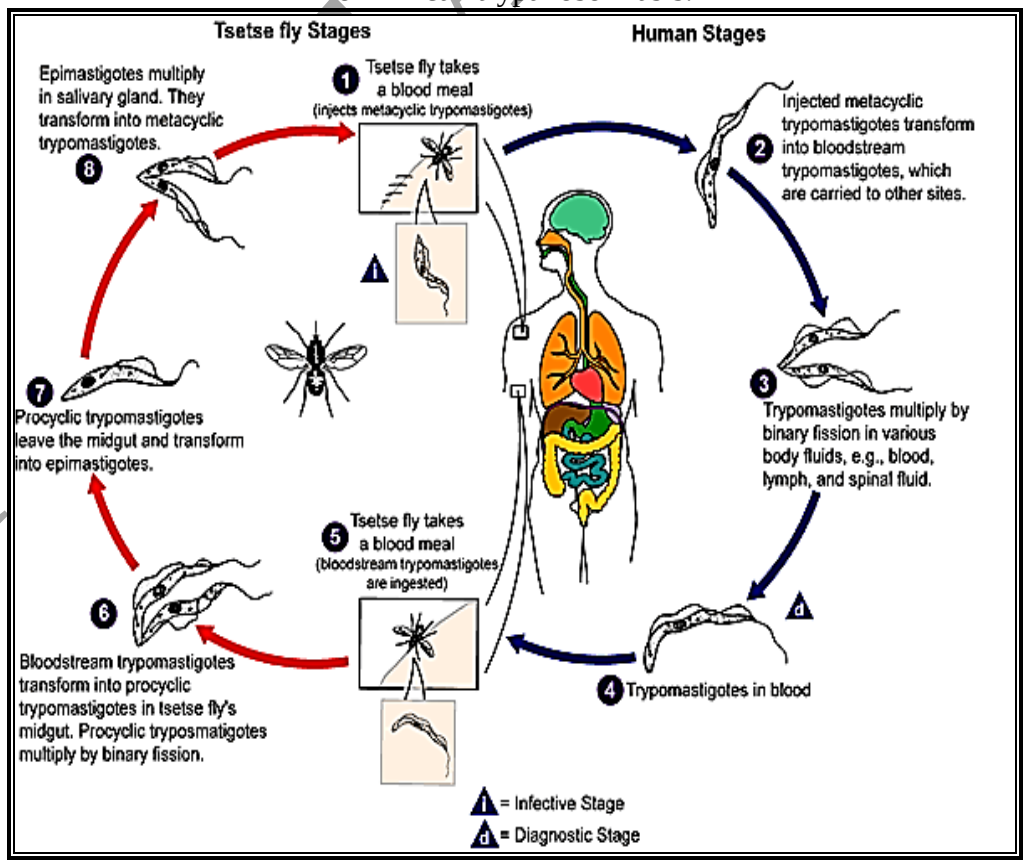
The cycle starts when the fly takes its blood meal and injects the infective stages. A week later, it will arrive to the blood and start in multiplication.

Trypanosomes considered coelozoic parasites because it lives in the blood, lymph, spaces in the lymph glands and the spleen, the liver and many other organs. It is also transmitted through the placenta (congenital transmission).

A. Invertebrate host:

When the insect takes its blood meal from the infected host, the trypanosomes enters with the sucking blood to the digestive tract of the insect where the parasite will start in multiplication in the mid and hind gut. For a period of 10 days, a large number of parasites will be formed, then the parasites will migrate forward until they arrive the salivary glands and transform to epimastigotes and multiply again, then produce the infective metacyclic trypanosomes. The development cycle extends 20-30 days in 24˚C. The development within the fly requires approximately 3 weeks.

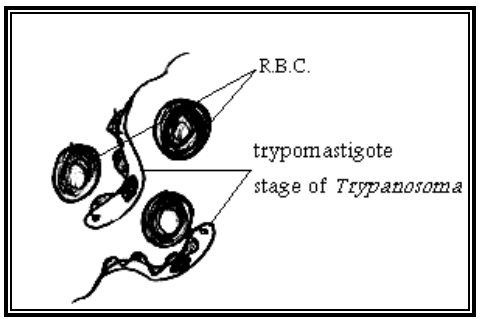
Glossina palpalis (tse-tse fly) the vector of African trypanosomiasis.



"Diagram for the life cycle of African Trypanosomes of man"

1. *Trypanosoma gambiense*

It causes Gambian Trypanosomiasis or called African sleeping sickness. In the first stage of the infection, the parasite present in the blood and lymph nodes whereas after the brain infection the parasite will be present in the cerebrospinal fluid. Its multiplication by longitudinal binary fission.



Trypomastigote stage in the blood.

Pathology and Symptomatology

After the insect's bite, an inflammation reaction around the wound of the bite will occur, almost boil-like, red or dark. After many days, the patient suffering from a fever and headache for irregular periods for many weeks or months, accompanied with lymph node enlargement and extreme weakness, decrease in the resistance to other diseases, almost myocarditis may be present and skin rashes in the primary stages. yet, sometimes there is no symptom except the trypanosomal fever.

Within 6-14 days (the incubation period) the parasite appears in circulating blood and cerebrospinal fluid where the symptoms of sleeping sickness appear; at this stage common complaints appear. Later dyspnea, precordial pain and anemia occurs. The patient may die from fulminating toxemia.

Diagnosis

By the demonstration of the trypanosomes in the chancre fluid, blood, tissue fluid aspirated from enlarged lymph nodes, bone marrow biopsy or, in the late stages of infection, cerebrospinal fluid. A wet preparation should be examined for the motile trypanosomes; in addition, a smear should be fixed and stained with Giemsa.

IgM measurement, fluorescent antibody test are serological methods used also but antibody detection has sensitivity and specificity that are too variable for clinical decisions.

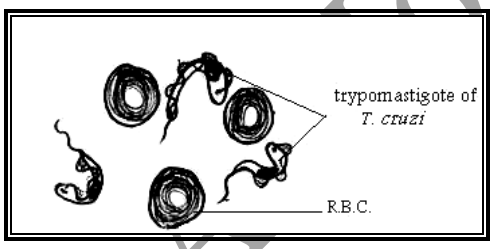
Treatment: Pentamidine and suramin.

2. *Trypanosoma rhodesiense*

It causes Rhodesian Trypanosomiasis or called East African sleeping sickness. Life cycle as similar as the life cycle of ***T. gambiense***. This type of disease as similar as the Gambian type in the symptoms and the progression of the disease except that the Rhodesian type is faster in its development, more acute than the first type, causes the death in 3-4 months after the infection, and many times the disease does not associated with lymph glands megally, which considers a distinguishable sign in the Gambian type.

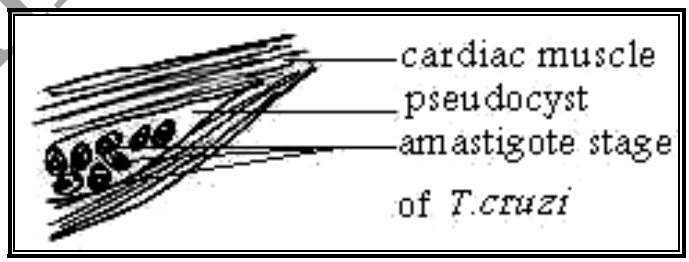
3. *Trypanosoma (Scizotrypanum) cruzi*

It causes Chagas' disease or American type Trypanosomiasis. The parasite has C or U or S-shaped. Trypomastigote is the only stage found in the blood of an infected person.



Trypomastigote stage in the blood.

In its intracellular phase it is typical amastigote. The epimastigote stage is not seen in humans but can be found in the midgut of the **triatomes** that have ingested the trypomastigotes from an infected host. The parasite multiplication occurs just in the tissues by binary fission, it forms pseudocysts by their accumulation.



Section in the cardiac muscle.

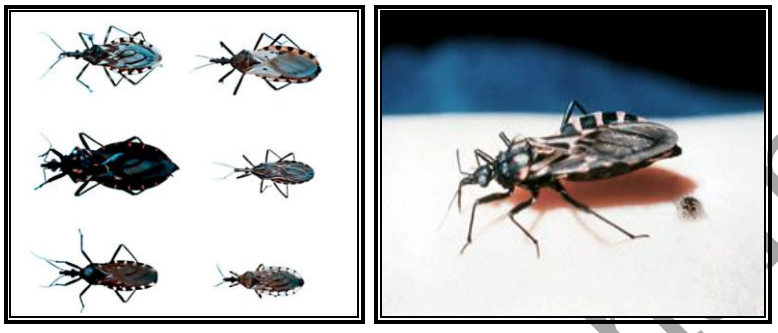
Life cycle

A. Invertebrate host:

Individuals of Triatomatidae, mostly ***Triatoma infestans****. The adult, larva and nymph, all of them can transmit the infection by sucking the blood from the infected

mammals which contain the trypomastigote or amastigote stage. In the midgut of the insect this parasite will transform to epimastigote and pass through binary fissions then it transforms to the infective stage which called metacyclic trypanosomes and migrate to the hindgut to exit with the feces of the insect when it takes its blood meal from the vertebrate host.

* Hint: There are other common vector species for this type of Trypanosomiasis belong to the genera ***Triatoma***, ***Rhodinius***, and ***Panstrongylus***.



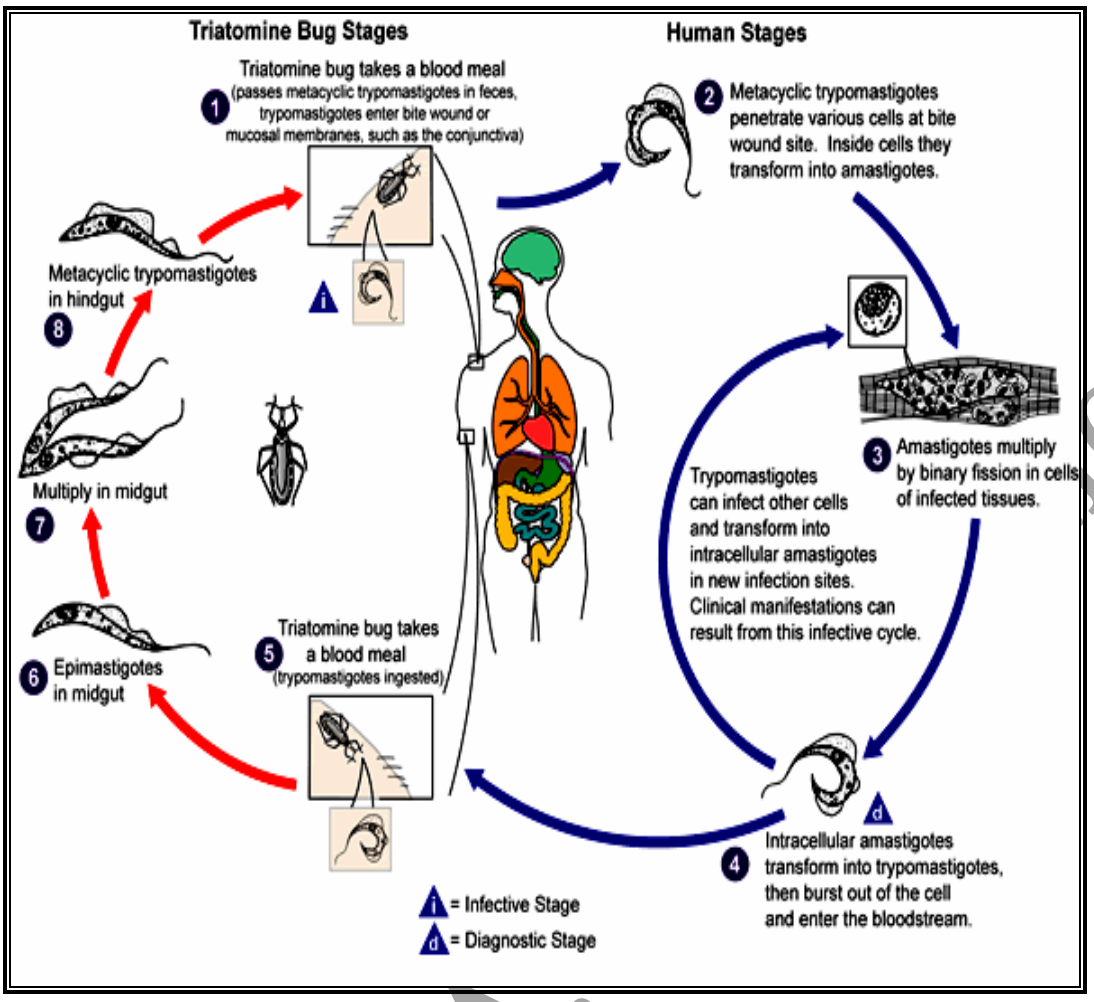
Triatome bug (the vector of the American type trypanosomiasis).

B. Vertebrate host:

The human becomes infected while bug bites during the sleep around the lips and eyes, therefore the insect called **[kissing bug]**.

During or following the blood meal, the infected bugs discharge the feces containing the infective metacyclic trypomastigotes which enters the host through the bite wound, so, this method called **contaminative method**, or it enters through the intact mucous membranes, ex. conjunctiva and mouth.

The parasites are engulfed by the macrophages and sometimes arrives to the muscles, endothelial cells of the lungs, the liver and lymph nodes and become amastigote then transform to spindle flagellated shapes and transform to promastigote, epimastigote and finally trypomastigote after 4 days and disrupt the cell and enter the blood, lymph stream and other tissues where the cycle restarts as long as the host alive. The infection can also be transmitted through blood transfusions, organ transplantation, transplacentally and in laboratory accidents.

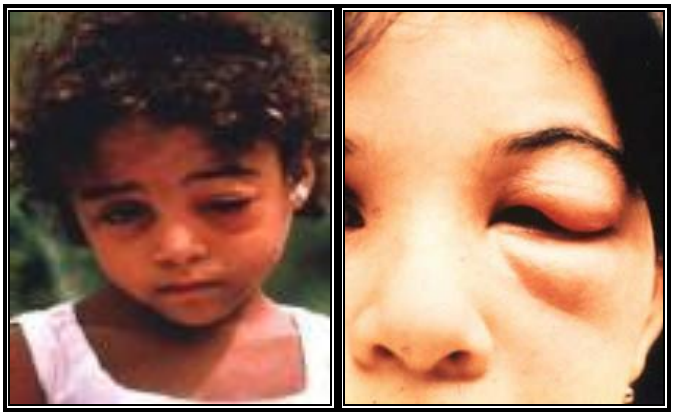


"Diagram for the life cycle of American Trypanosomiasis of man"

Symptomatology

When first generation emerges from the macrophages, they cause a localized tissue reaction and acute regional lymphadenitis. The incubation period ranges from 5-12 days, usually. The symptoms characterized by unilateral painless, erythromatous palpebral edema, at times extending to the entire side of the face accompanied by enlarged preauricular or submaxillary lymph nodes, conjunctivitis and dacryoadenitis.

Less frequently is the **chagoma**, an erythematous, itching but painless infiltration of the dermis with central desquamation and rarely ulceration of the skin. The **chagoma** is localized on exposed parts of the sleeping individuals**.** Through the blood, the infection may spread, causing signs and symptoms such as fever, hepatosplenomegaly, generalized lymphadenitis and edema, muscular pain, vomiting and diarrhea, bronchitis and myocarditis of variable intensity, anemia, leukocytosis (lymphocytosis and monocytosis) and increased IgM levels.



Unilateral conjunctivitis and orbital edema.

Diagnosis

1. During the early stage: the blood films, searching about trypomastigotes mainly. During acute febrile: muscle biopsy (amastigotes).

Trypomastigotes may be seen in cerebrospinal fluid (CSF) in central nervous system infections; also, the amastigote stage parasite may be seen in histopathology specimens from affected organs.

2. During the late stage, the parasitemia is low. The best method is **Xenodiagnosis**.

Xenodiagnosis: is the diagnostic method by which clean ***Triatoma*** are allowed to bite the patient, the flagellates are found in the bug's feces usually 30 days later.

3. It can also be diagnosed by serological tests such as CF, IHM, ELISA, IF.

Treatment: Nifurtimox.