Blood and Tissue Flagellate Trypanosomes

Introduction

Trypanosomes are protozoan parasites belonging to the subphylum *Mastigophora*. The trypanosomaes of medical importance are

1. **Salivarian group** : *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* which cause African Trypanosomiasis in humans. The disease is also known as African sleeping sickness.

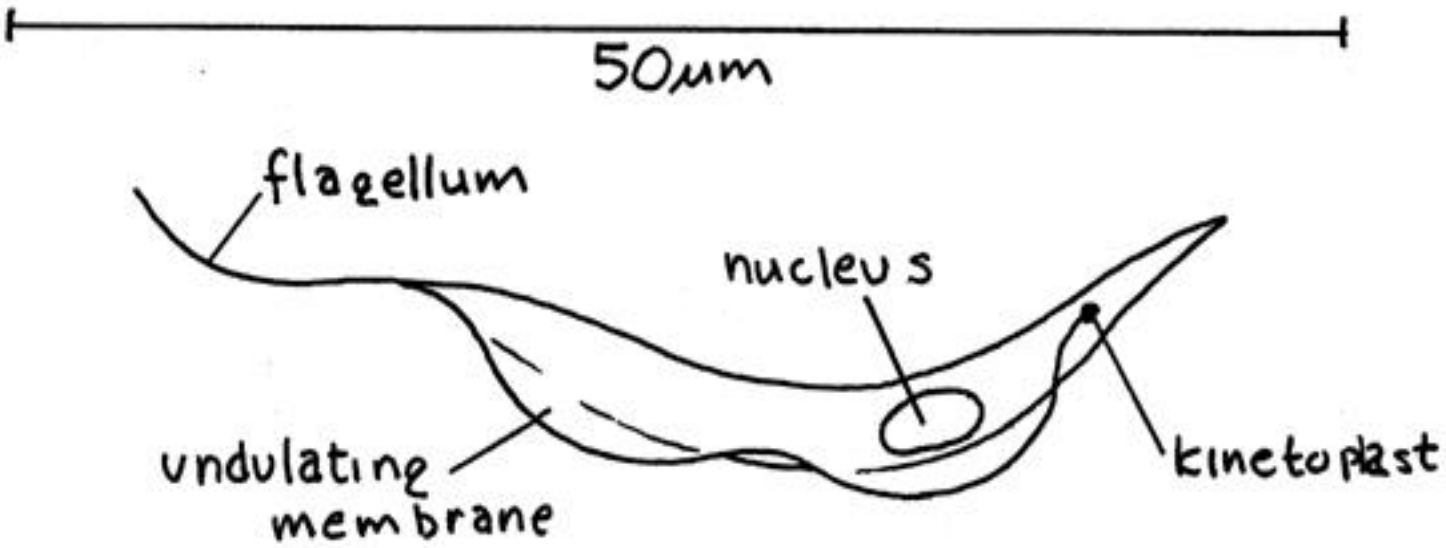
T. brucei belongs to the **salivarian group** of trypanosomes which develop mid gut in the vector and transmission is by inoculation when the vector feeds. The vector is the *Tse-Tse* fly. -*T. b. rhodesiense* and *T. b. gambiense* are indistinguishable under a microscope

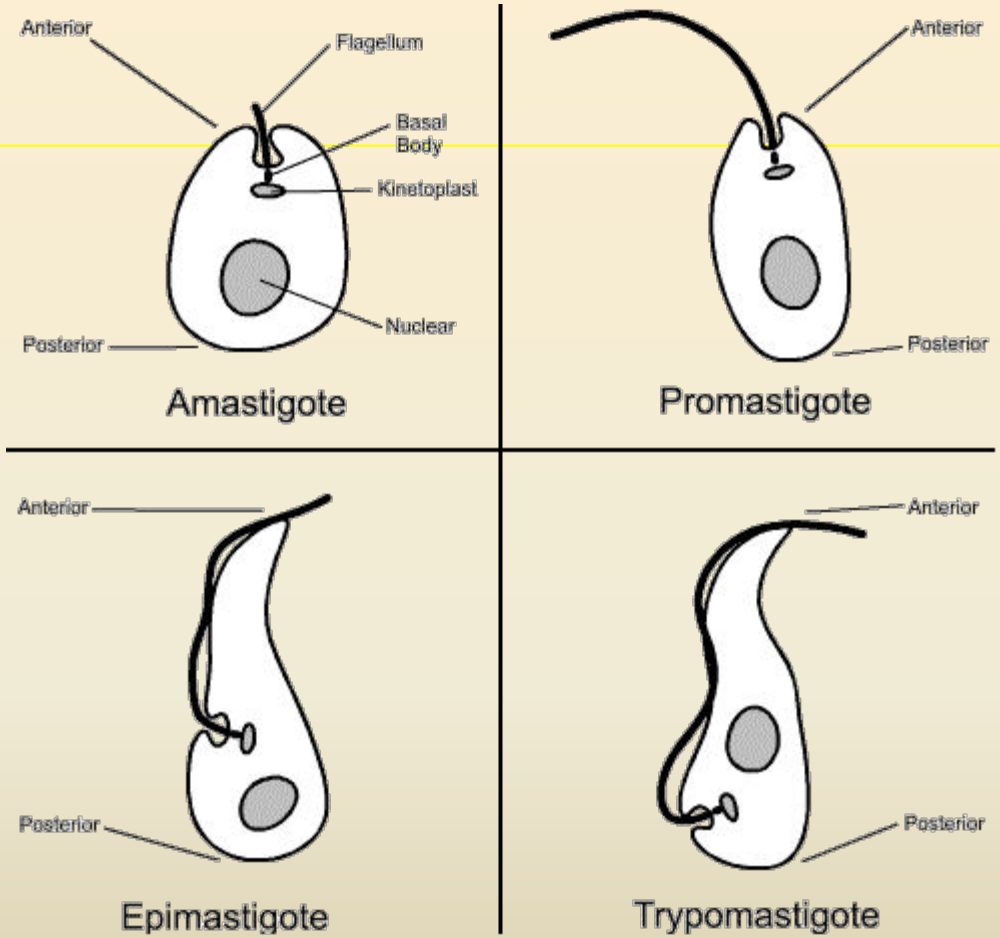
Stercorarian group: *Trypanosoma cruzi* which causes American trypanosomiasis, or Chagas disease (Carlos Chagas 1907). *T. cruzi* belongs to the **stercorarian group** of trypanosomes which develop in the hindgut of the vector and transmission is by faecal contamination after the vector bites. The vector is the Reduvid bug.

.2

Morphology

Trypanosomes are haemoflagellates i.e. actively motile flagellated parasites that live in the blood and lymph. The single flagellum arises from the Kinetoplast which is situated posterior to the nucleus. The flagellum runs the length of the body along the undulating membrane and usually beyond it as an anterior free flagellum. The flagellate in humans with the kinetoplast positioned posterior to the nucleus is called a TRYPOMASTIGOTE or simply a trypanosome. *T. cruzi* has a non flagellate form found in tissue cells and this intracellular form is called an AMASTIGOTE.


50µm
flagellum
nucleus
undulating membrane
kinetoplast


Anterior
Flagellum
Basal Body
Kinetoplast
Nuclear
Posterior
Amastigote
Anterior
Posterior
Promastigote
Anterior
Posterior
Epimastigote
Anterior
Posterior
Trypomastigote

- *Trypanosoma brucei* is not killed by the immune system because it has a Variable Surface Glycoprotein (VSG) coating. The coating makes its cell membrane very thick and hard to recognize. It also changes frequently its structure to always keep ahead of the immune response.

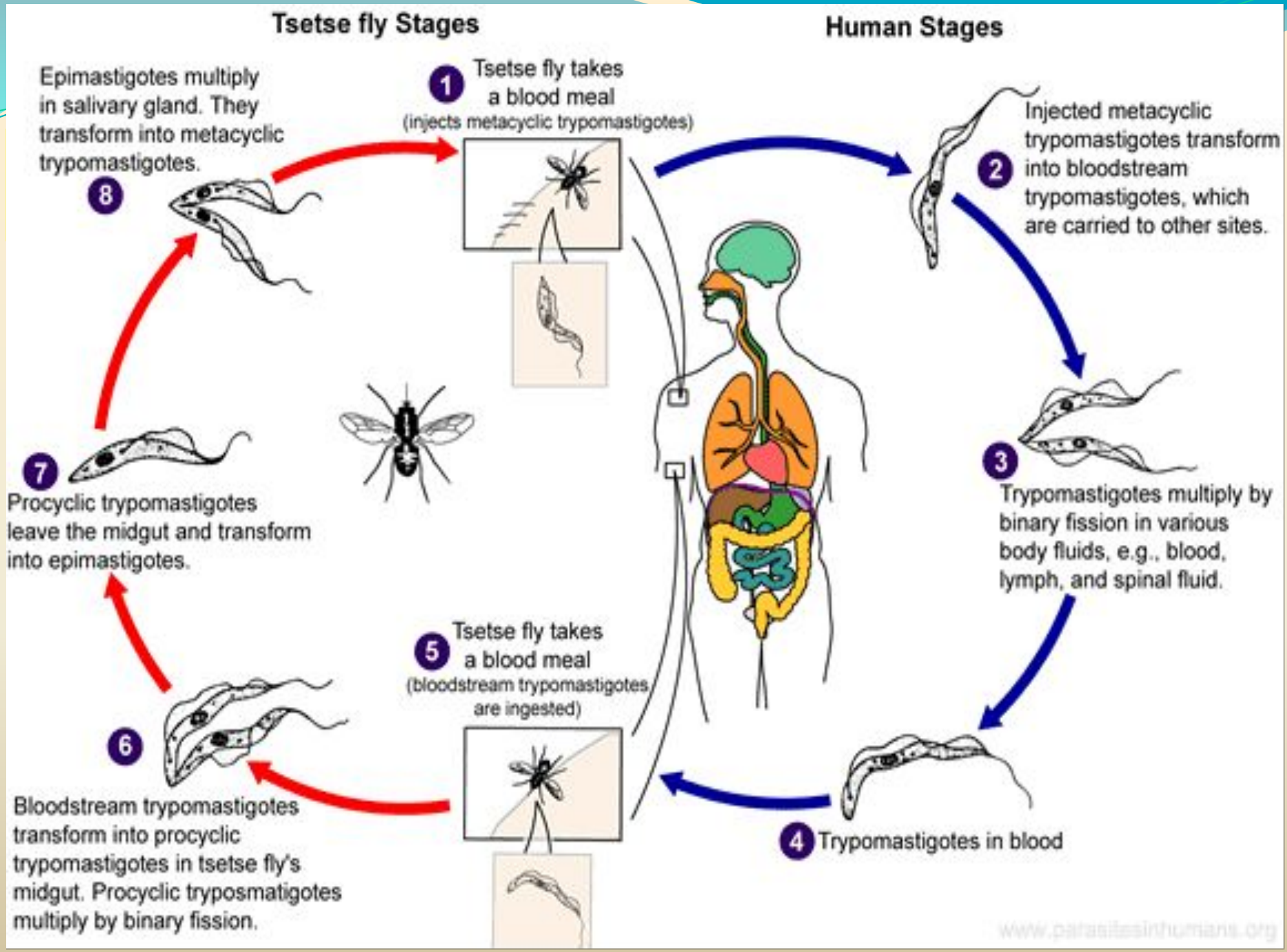
- - The two properties of the VSG coat that allow immune evasion are:
- Defensive - the dense nature of the VSG coat prevents the immune system of the mammalian host from accessing the plasma membrane or any other invariant surface epitopes (such as ion channels, transporters, receptors etc.) of the parasite.
- Periodic antigenic variation - the VSG coat undergoes frequent stochastic genetic modification - 'switching' - allowing variants expressing a new VSG coat to escape the specific immune response raised against the previous coat.

African Trypanosomiasis

Life cycle

- Metacyclic (infective) trypomastigotes are inoculated through the skin when a tsetse fly takes a blood meal. The parasites develop into long slender trypomastigotes which multiply at the site of inoculation and later in the blood, lymphatic system and tissue fluid.
- The trypomastigotes are carried to the heart and various organs and in later stages of infection invade the CNS.
- Trypomastigotes are taken up by the tsetse fly when it sucks blood (male and female).
- The parasites develop in the midgut of the fly and multiply.
- From about 2-3 weeks the trypomastigotes move to the salivary glands transforming through epimastigotes into metacyclic trypomastigotes. The tsetse fly remains infective for life - about 3 months. *T. b. rhodesiense* infects many animals as well as humans whereas *T. b. gambiense* is infective to fewer animals. In Africa the disease causes a lot of cattle wasting because of the infection rate.

- The reservoirs of infection for these vectors are exclusively human in West African trypanosomiasis (*T. b. gambiense*). However, East African trypanosomiasis is a zoonotic infection with animal vectors (*T. b. rhodesiense*) .


Tsetse fly Stages
Human Stages
Epimastigotes multiply in salivary gland. They transform into metacyclic trypomastigotes.
8
1 Tsetse fly takes a blood meal
(injects metacyclic trypomastigotes)
2 Injected metacyclic trypomastigotes transform into bloodstream trypomastigotes, which are carried to other sites.
3 Trypomastigotes multiply by binary fission in various body fluids, e.g., blood, lymph, and spinal fluid.
4 Trypomastigotes in blood
5 Tsetse fly takes a blood meal
(bloodstream trypomastigotes are ingested)
6 Bloodstream trypomastigotes transform into procyclic trypomastigotes in tsetse fly's midgut. Procyclic tryposmatigotes multiply by binary fission.
7 Procyclic trypomastigotes leave the midgut and transform into epimastigotes.

Signds and Symptoms

- **Physical**
- Stage 1 (early, or hemolymphatic, stage)
- Indurated chancre at bite site
- Skin lesions (trypanids) in light-skinned patients
- Lymphadenopathy:
- Axillary and inguinal lymphadenopathy are more common in patients with East African trypanosomiasis.
- Cervical lymphadenopathy is more common in patients with West African trypanosomiasis.
- The classic Winterbottom sign is clearly visible (ie, enlarged, nontender, mobile posterior cervical lymph node).
- Fevers, tachycardia, irregular rash, edema, and weight loss
- Organomegaly, particularly splenomegaly (*T brucei gambiense* African trypanosomiasis)

- In the early stages of the disease there is a high irregular fever with shivering, sweating and an increased pulse rate. The lymph glands near the bite often become swollen, **Gambiense the glands at the back of the neck and Rhodesiense usually the glands under the jaw are affected**.

- Enlarged spleen edema of the eyelids, face and sleeplessness are features as the disease progresses.

- Stage 2 (late, or CNS, stage) Persistent

- headaches (refractory to analgesics)
- Daytime somnolence followed by nighttime insomnia
- Behavioral changes, mood swings, and, in some patients, depression
- Loss of appetite, wasting syndrome, and weight loss
- Seizures in children (rarely in adults)
- Fevers, tachycardia, irregular rash, edema

In the late stages of the disease the trypanosomes invade the CNS giving symptoms of meningoencephalitis, mental dullness, apathy, excessive sleeping and incontinence.

The CSF usually contains mononuclear cells and a few trypanosomes may be detected. CSF protein is raised. If untreated, coma develops and finally death. **Such signs are more commonly seen with Gambiense than in Rhodesians in which patients often die before these symptoms develop fully.**

- African trypanosomiasis is a wasting disease which is usually fatal unless treated. **East African trypanosomiasis**(*T.b. rhodesians)* is more acute and progresses .quicker than **West African trypanosomiasis** (*T.b.gambians)*

Pathology and Immunology

An exact pathogenesis of sleeping sickness is not known, although immune complexes and inflammation have been suspected to be the mechanism of damage to tissues

- The gastrointestinal system is also affected. Kupffer cell hyperplasia occurs in the liver, along with portal infiltration and fatty degeneration.
- Hepatomegaly is rare. More commonly in East African trypanosomiasis, a pancarditis affecting all heart tissue layers develops secondary to extensive cellular infiltration and fibrosis.
- Arrhythmia or cardiac failure can cause death prior to the development of CNS manifestations.
- CNS problems include perivascular infiltration into the interstitium in the brain and spinal cord, leading to meningoencephalitis with edema, bleeding, and granulomatous lesions.

- The immune response against the organism **does help** to eliminate the parasite but it is not protective, since the parasite has a unique ability of altering its antigens, the VSG. Consequently, there is a cyclic fluctuation (changeability) in the number of parasites in blood and lymphatic fluids and each wave of parasite represents a different antigenic variant
- The parasite causes polyclonal expansion of B lymphocytes and plasma cells and an increase in total IgM concentration. It stimulates the reticuloendothelial function. It also causes severe depression of cell mediated and humoral immunity to other antigens .

- **Laboratory Diagnosis**

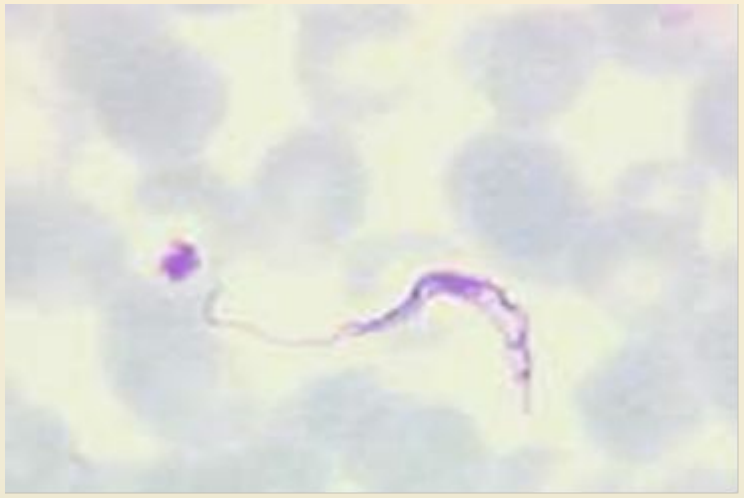
a. Examination of blood for trypanosomes (thick/thin) and Buffy coat

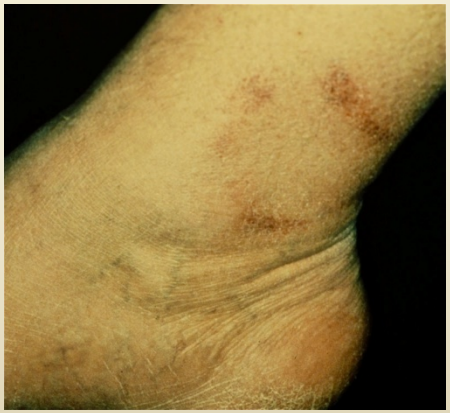
b. Aspirates from enlarged lymph glands - .trypanosomes

c. CSF for trypanosomes, cells (Morula), raised .protein , IgM and motile trypanosomes

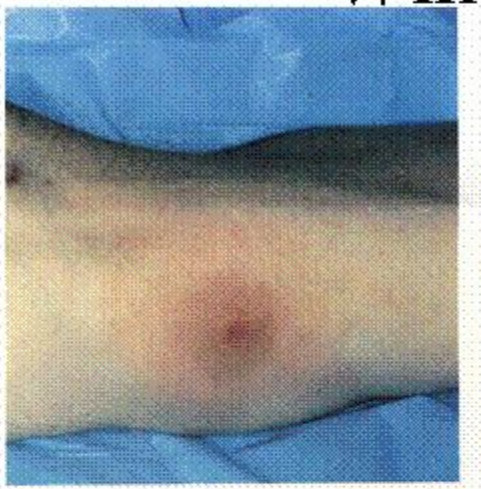
d. Serum for anti trypanosomal antibodies - tests .available for *T. b. gambiense* (IFAT, ELISA, CFT)

- **Treatment and Control**
- The blood stage of African trypanosomiasis can be treated with reasonable success with **Pentamidine isethionate or Suramin**. These drugs have been reported also to be effective in prophylaxis.
- Cases with CNS involvement should be treated with **Melarsoprol**, an organic arsenic compound.
- The most effective means of prevention is to avoid contact with tsetse flies. Vector eradication is impractical due to the vast area involved.
- Immunization has not been effective due to antigenic variation.





Local Chancre and Winterbottom's sign



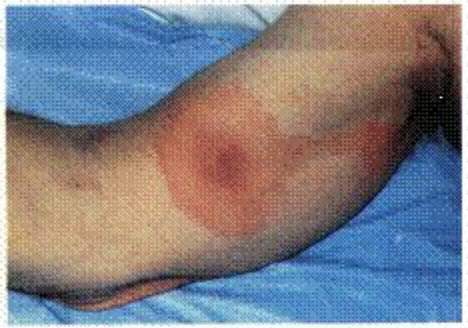
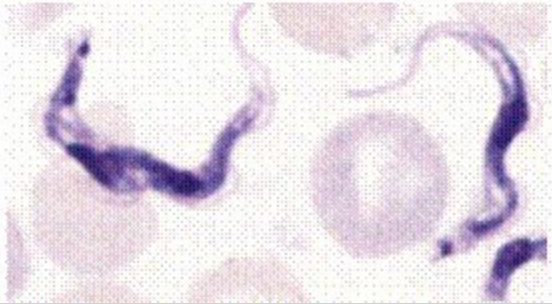


Figure 1 (Panosian et al.). Pretreatment photograph of medial aspect of patient's arm, showing nonulcerated, indurated nodule at presumed trypanosome inoculation site.

Figure 2 (Panosian et al.). Photograph of trypanosomal chancre following treatment with one dose each of pentamidine and suramin. Note increased erythema, with extension into the axilla.

Trypomastigotes in the lymphnode

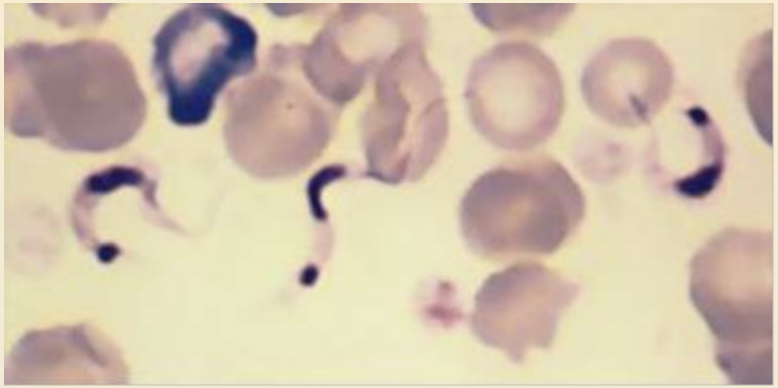


Late Stage (Encephalitic Stage)

- Finally, the late stage leads the host to death.
- The host enters a terminal coma, or "sleep," giving the disease its name: "sleeping sickness."

West African Sleeping Sickness

- Initially a skin lesion with swelling
- Winterbottom's sign – swelling of cervical lymph nodes
- Eventually parasite enters CNS
- CNS involvement often results in wasting condition.
- Untreated patients lapse into stupor, convulsions, death.



American Trypaniomasis (Chagas disease)

- Chagas disease is caused by a protozoan parasite named *Trypanosoma cruzi*.
- Infection of humans occurs when an insect vector (mainly *Triatominae* or kissing bugs) deposits feces that contains the parasites on human skin.
- The parasites then enter the mammalian (human) host through the bug bite, or breaks in the skin or conjunctiva.
- Occasionally, the parasites enter through mucosal cells when ingested or inhaled.
- The bugs often deposit feces near the eyes and lips; when the parasites enter the skin, swelling and redness (Multiplication of *T. cruzi* at the site of infection can produce an inflamed swelling chagoma) often develop.

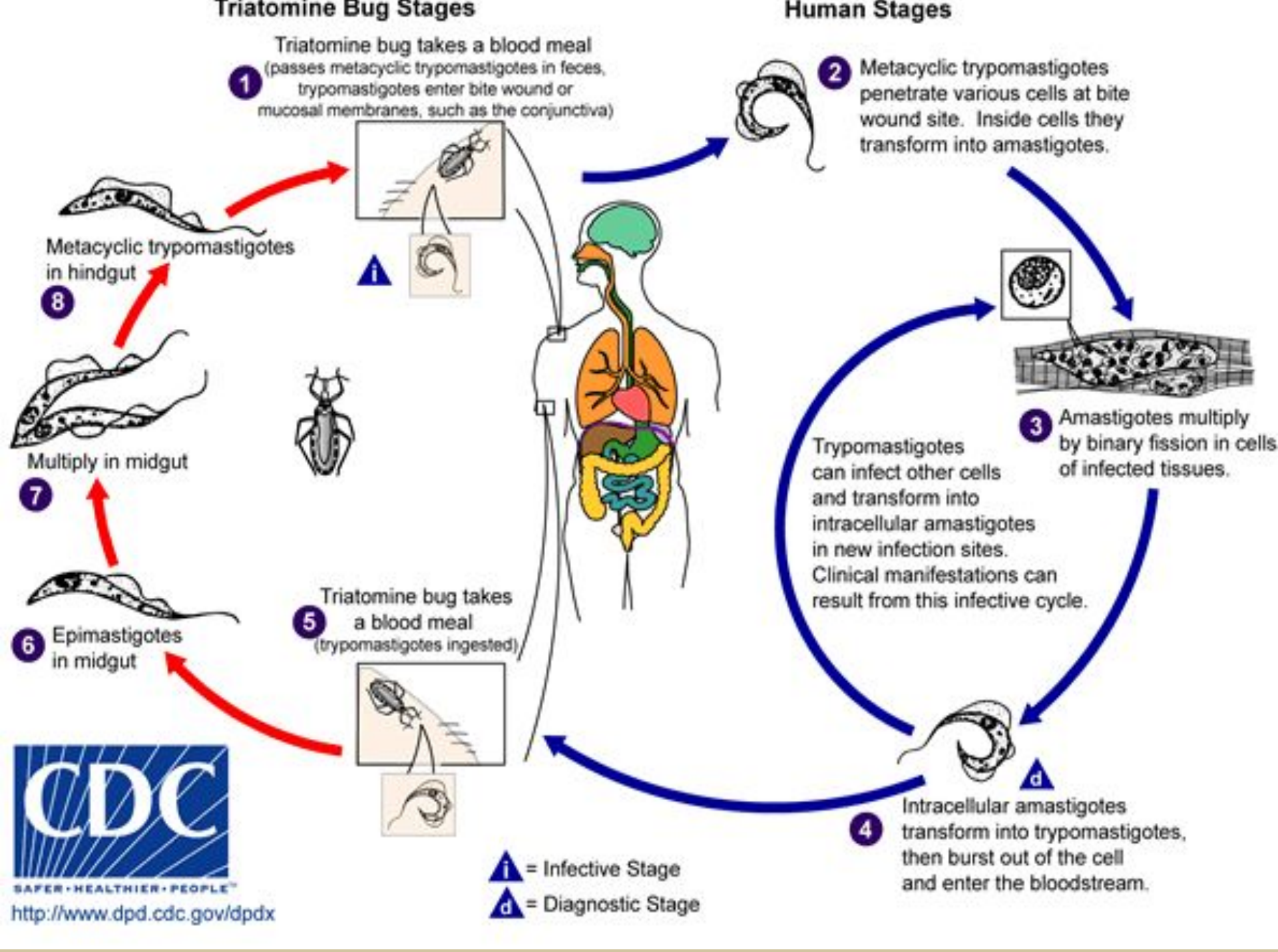
- The term "kissing bugs" comes from the appearance of these symptoms that resemble skin changes that occur with prolonged kissing. In some individuals, the parasites eventually go into the bloodstream and lodge in various organs, multiply, and eventually cause chronic symptoms such as cardiac arrhythmias, poor gastrointestinal motility, or death.

Life cycle

- The life cycle of *T. cruzi* is complex; it has multiple developmental stages in both the insect vector (*Triatominae* bugs and also termed *triatomine* bugs) and mammalian (human and animal such as dog or cat) hosts.
- Metacyclic trypomastigotes are deposited in faeces on the skin as the triatomine bug (Reduvid)feeds.
- The bug usually bites round the edges of the mouth and eyes. The trypomastigotes are either rubbed into the skin by scratching the irritated area or penetrate the conjunctiva or membranes of the nose and mouth.
- **Trypomastigotes become amastigotes in localised RE cells and multiply.**

- The amastigotes develop into trypomastigotes which are released into the blood when the cell ruptures.
- No multiplication of the parasite takes place in the blood in its trypomastigote stage.
- By way of the blood and lymphatic system the trypomastigotes reach tissue cells especially **heart muscle, nerves, skeletal muscle and smooth muscle of the gastrointestinal system**. The trypomastigotes become amastigotes and multiply forming pseudocysts.

- One trypomastigote may produce 500 amastigotes within a single pseudocyst in as little as 5 days.
- Within the pseudocyst some amastigotes become elongated and develop first into epimastigotes and then trypomastigotes. When the cell ruptures the trypomastigotes are released into the blood and continue to circulate whilst others invade further tissue cells.
- The life cycle completes when a triatomine bug vector ingests circulating trypomastigotes. In the vector the trypomastigotes transform and develop into epimastigotes, multiply by binary fission in the gut of the bug. After about 10 - 15 days, metacyclic trypomastigotes are formed and can be found in the hindgut of the bug.


Triatomine Bug Stages
Human Stages
Triatomine bug takes a blood meal
(passes metacyclic trypomastigotes in feces, trypomastigotes enter bite wound or mucosal membranes, such as the conjunctiva)
1
2
Metacyclic trypomastigotes penetrate various cells at bite wound site. Inside cells they transform into amastigotes.
Metacyclic trypomastigotes in hindgut
8
i
3
Amastigotes multiply by binary fission in cells of infected tissues.
Trypomastigotes can infect other cells and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle.
Multiply in midgut
7
Triatomine bug takes a blood meal
(trypomastigotes ingested)
5
6
Epimastigotes in midgut
d
4
Intracellular amastigotes transform into trypomastigotes, then burst out of the cell and enter the bloodstream.
CDC
SAFER · HEALTHIER · PEOPLE™
http://www.dpd.cdc.gov/dpdx
i = Infective Stage
d = Diagnostic Stage

Signs and symptoms

- The symptoms of Chagas disease can be quite variable and range from no symptoms to severe. Most individuals who get the acute-phase symptoms have them resolve spontaneously in about three to eight weeks. The first symptoms, when present in the **acute phase,** may include some of the following:
- Swelling and/or redness at the skin infection site (termed chagoma)
- Rash
- Swollen lymph nodes
- Fever
- Head and body aches
- Fatigue
- Nausea, vomiting, and/or diarrhea
- Liver and/or spleen enlargement
- **Romaña sign** (unilateral painless edema of tissues around the eye). Mltiplication of *T.cruzi* in the eye then the conjunctiva becomes inflamed
- Occasionally, acute infections show chronic symptoms if the patient is severely immunocompromised.

- **Symptoms of chronic Chagas disease**
- vary according to the organs most affected;The symptomatic (determinate) chronic stage affects the nervous system, digestive system and heart.
- About two-thirds of people with chronic symptoms have cardiac damage, including dilated cardiomyopathy, which causes heart beat abnormalities and may result in sudden death.
- About one-third of patients go on to develop digestive system damage, resulting in dilation of the digestive tract (megacolon and megaesophagus), accompanied by severe weight loss. Swallowing difficulties (secondary achalasia) may be the first symptom of digestive disturbances and may lead to malnutrition.
- Up to 10% of chronically infected individuals develop neuritis that results in altered tendon reflexes and sensory impairment. Isolated cases exhibit central nervous system involvement, including dementia, confusion, chronic encephalopathy and sensitivity and motor deficits.

- In most cases, the heart or the gastrointestinal tract (or both) show the most serious symptoms. Chronic Chagas disease symptoms may include the following:
- Irregular heartbeats
- Palpitations
- Fainting (syncope)
- Cardiomyopathy
- Congestive heart failure
- Shortness of breath (dyspnea)
- Emphysema
- Stroke
- Chronic abdominal pain
- Chronic constipation
- Dilated colon
- Difficulty swallowing
- These symptoms are due to organ damage caused by the chronic presence of the parasites within the tissues of these organs.

- **Laboratory Diagnosis**
- **a.** Trypanosomes of *T. cruzi* in blood (early acute)
- 1) careful examination of fresh blood for motile trypanosomes as wet prep.
- 2) Capillary tube (microhaematocrit) concentration technique. Rapid and sensitive.
- b. Xenodiagnosis in chronic and sub acute (low parasitaemia).
- c. Blood culture for parasite when (b) not available.
- d. Serology - *T. cruzi* antibodies - distinguish between IgM (infant) and IgG (crossing placenta)
- **(1) IFAT** indirect fluorescence antibody test

- **Immunity**
- Unlike African trypanosomiasis, the antigenic variation is less common in *T.cruzi*
- infection. Therefore, the humoral and cellular immune responses function in the immune system.
- **Treatment**
- The drug of choice is nifurtimox. Alternative agents include allopurinol &
- benzimidazole.
- **Prevention**
- • Bug control, eradication of nests
- • Treating infected person & exclusion of donors by screening blood.
- • Development of vaccine.