

Phylum: sarcomastigophora

Subphylum: mastigophora

Class: zoomastigophora

Order: kinetoplastida


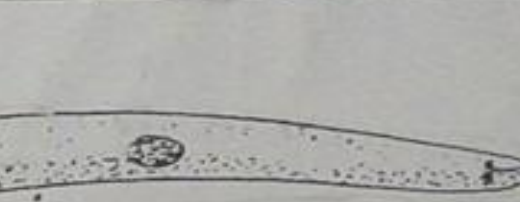

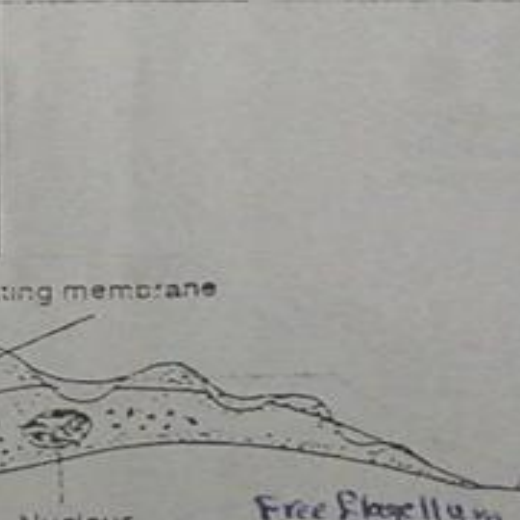
(Hemoflagellates)

Family: trypanosomatidae

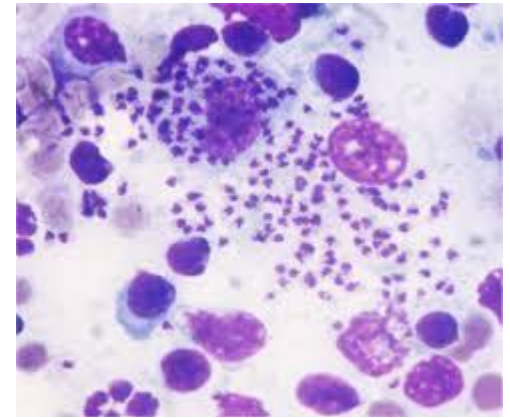
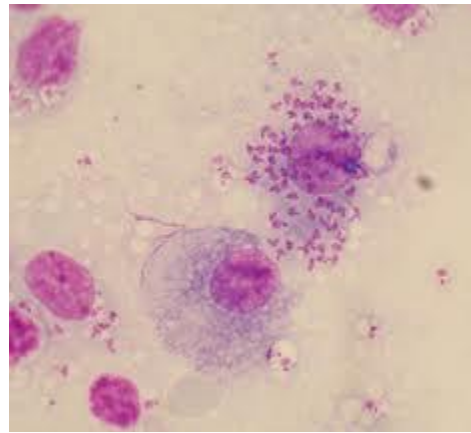
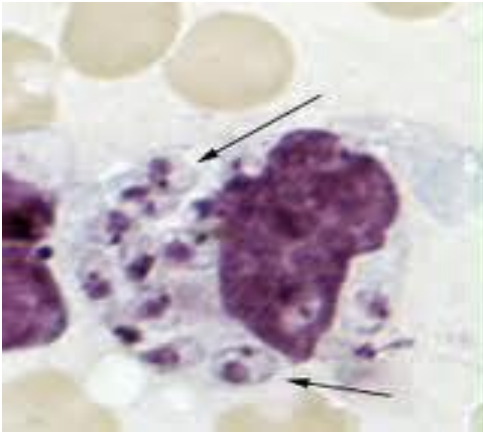
EX: *Leishmania Spp.*

Trypanosoma Spp.

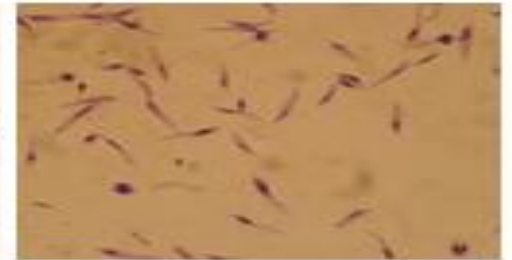
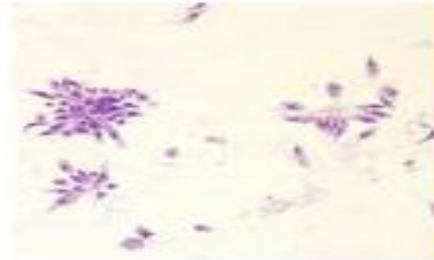
Morphological forms of haemoflagellates

Morphological form	Description	Form	Distribution of stages in haemoflagellates
Amastigote (non flagellar)	The cell is round or ovoid, without any external flagellum. The nucleus, kinetoplast and axoneme can be seen	 <p style="text-align: center;">Kinetoplast Nucleus Axoneme</p>	<i>T. cruzi</i> , <i>Leishmania</i> (intracellular in vertebrate hosts)
Promastigote	The cell is lanceolate in shape. Kinetoplast is at the extreme anterior end with free flagellum		Found In mid gut and in proboscis of vector (sandfly) and in culture of <i>leishmania</i> (infective form of <i>Leishmania</i>)
Epimastigote	This is more elongated kinetoplaste placed close to and in front of the nucleus with free flagellum and undulating membrane		Found in the of vector and in culture of <i>Trypanosoma</i>
Trypomastigote	Spindle shaped with a central nucleus and the kinetoplaste situated at the posterior end of the body. There is a long undulating membrane and a free flagellum	 <p style="text-align: center;">Undulating membrane Kinetoplast Nucleus Free Flagellum Velutin granule</p>	Found in the blood of the vertebrate host infected with trypanosomes, found in the vector for trypanosomes, named by (metacyclic trypomastigote) has short stumpy changeable shape. (infective form of <i>Trypanosoma</i>)

Amastigote

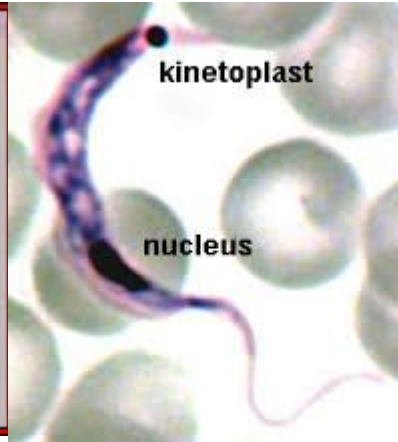
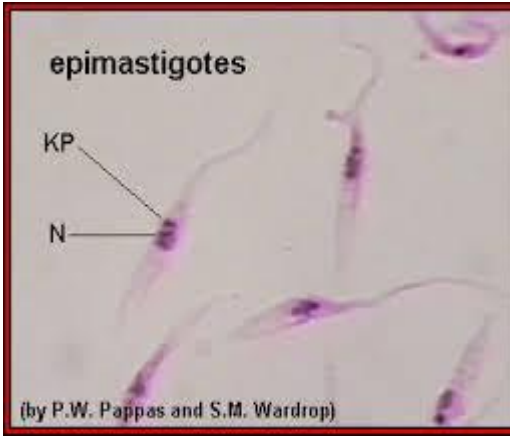


Promastigote

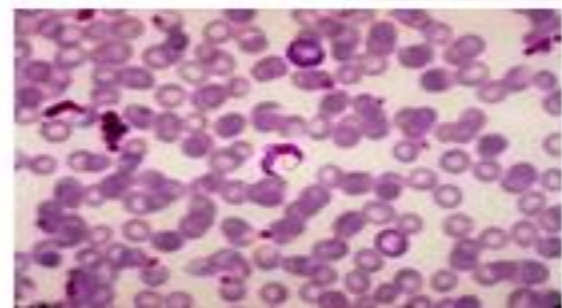
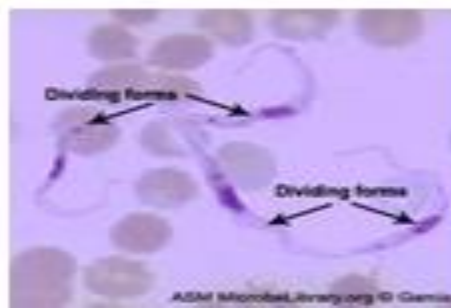
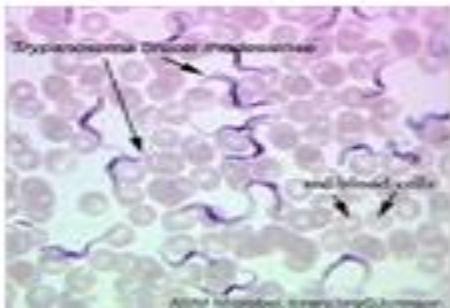
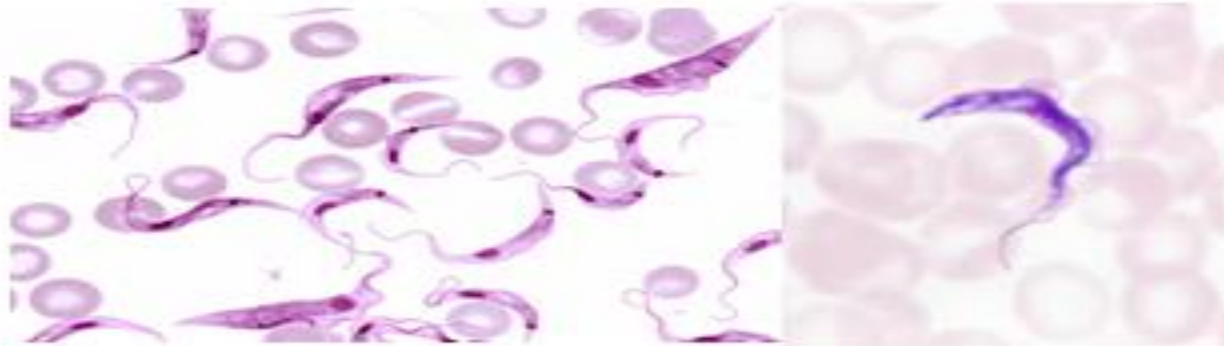


Epimastigote

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Trypomastigote



Leishmania: Three main species are involved

A- ***Leishmania donovani* complex** (causes visceral leishmaniasis or kala-azar or dum dum fever this is the most virulence form in the group)

B- ***Leishmania braziliensis* complex** (causes muco- cutaneous leishmaniasis , this is the intermediate virulent form)

C- ***Leishmania tropica* complex** (causes cutaneous leishmaniasis or oriental sore or Baghdad boil, this is the least virulent form)

Life cycle of *Leishmania* species :

These organisms require two hosts in their life cycle

1- Man and reservoir hosts (dogs , rodents etc) ,represent the definitive hosts.

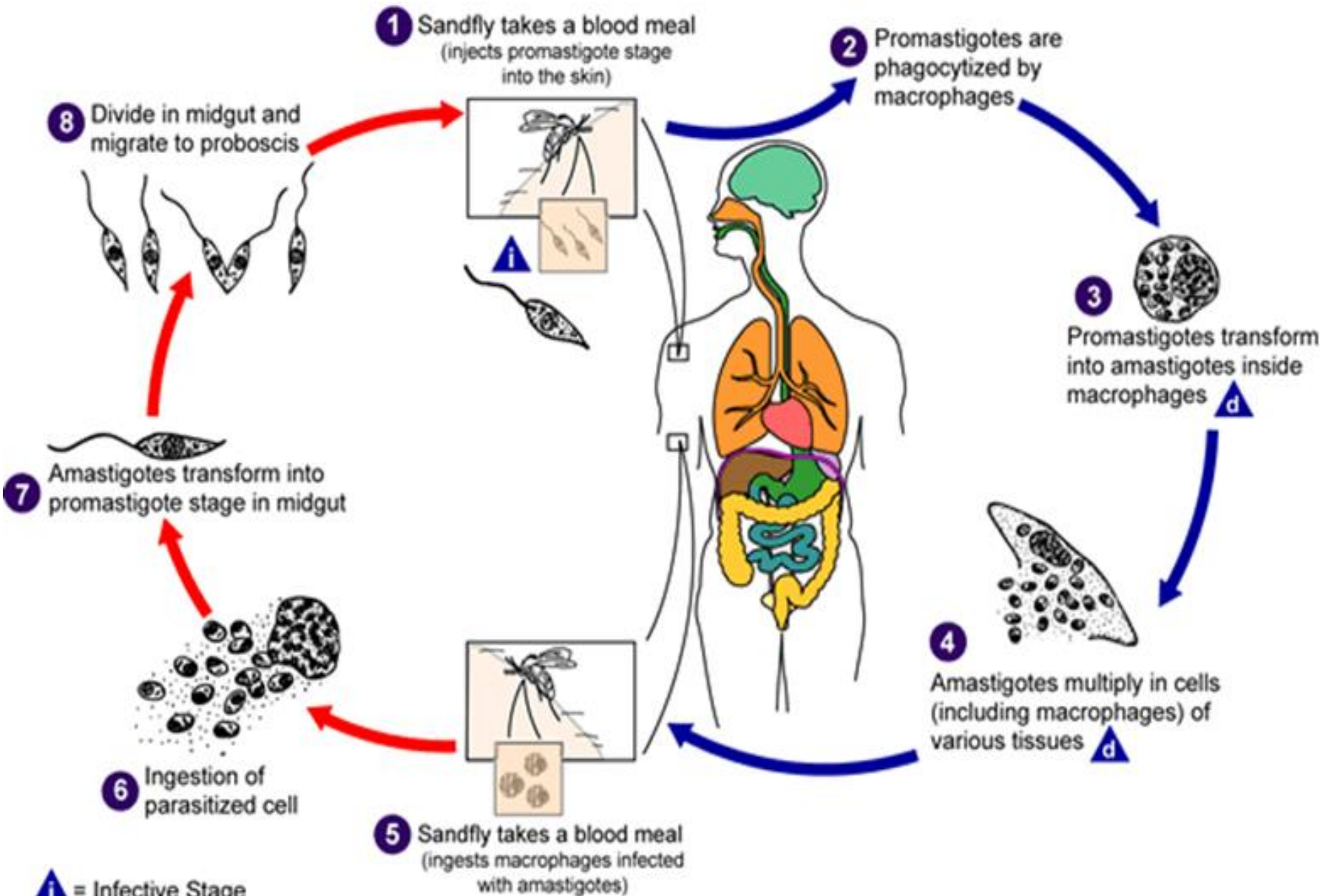
2- Blood sucking insect sandfly (phlebotomus),serve as intermediate host or vector

*Infective stage: Promastigote

*Diagnostic stage: Amastigote and promastigote

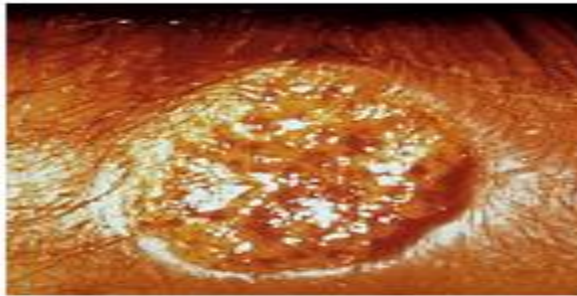
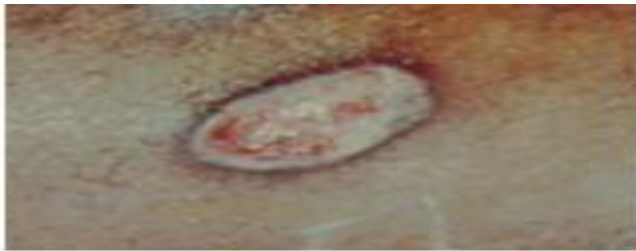
Sandfly Stages

Human Stages



i = Infective Stage

d = Diagnostic Stage



Wet lesion of CL (*leishmania tropica*)



Dry lesion of CL oriental sore ,or Baghdad boil (*leishmania tropica*)



Phlebotomus female



2. *Trypanosoma*:

(A) *T. gambiense* (African sleeping sickness).

(B) *T. rhodesiense* (African sleeping sickness).

(C) *T. cruzi* (South American trypanosomiasis-Chagas' disease)

T. brucei complex- African trypanosomiasis

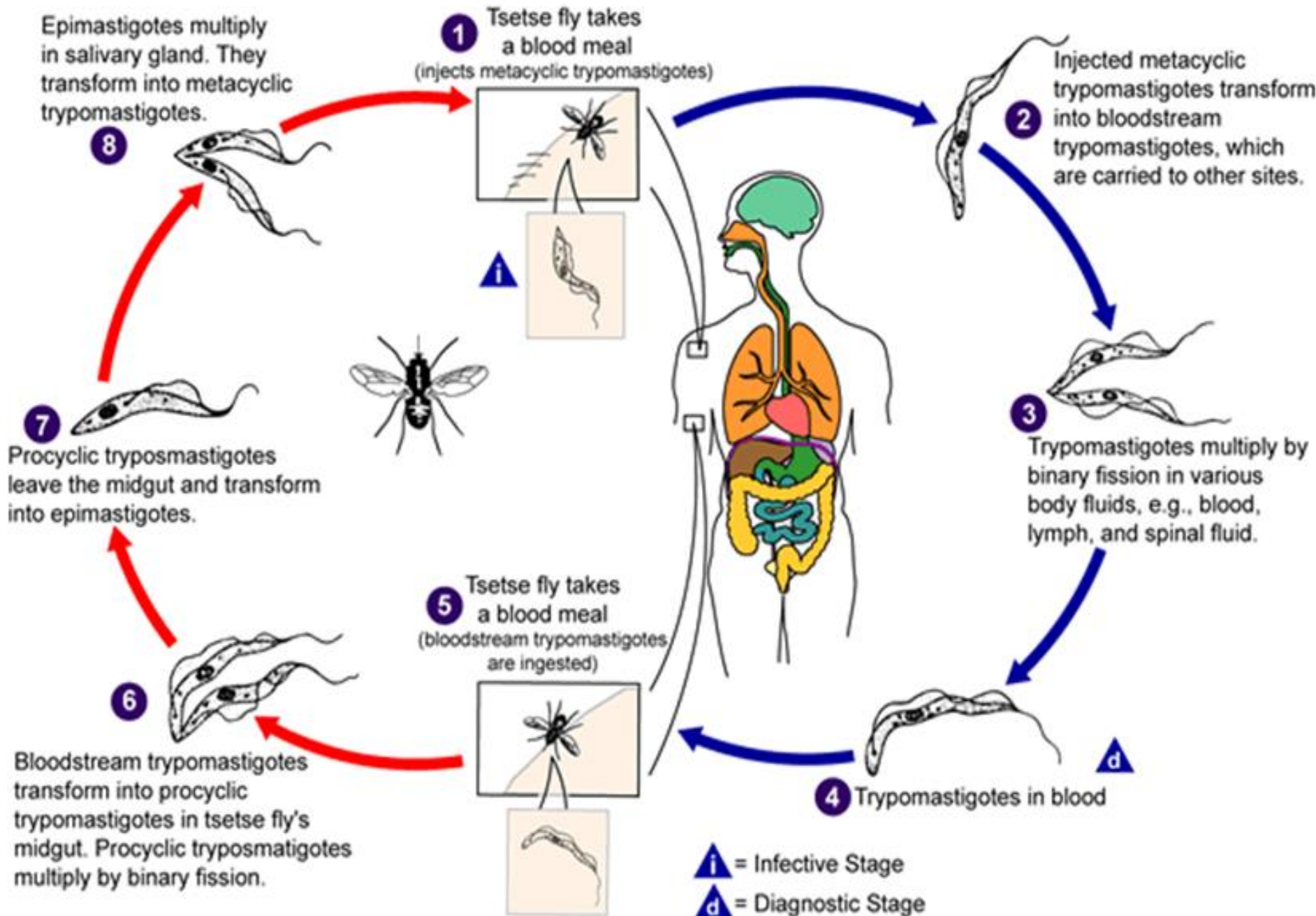
include: *Trypanosoma gambiense*

Trypanosoma rhodesiense

life cycle

Tsetse fly Stages

Human Stages



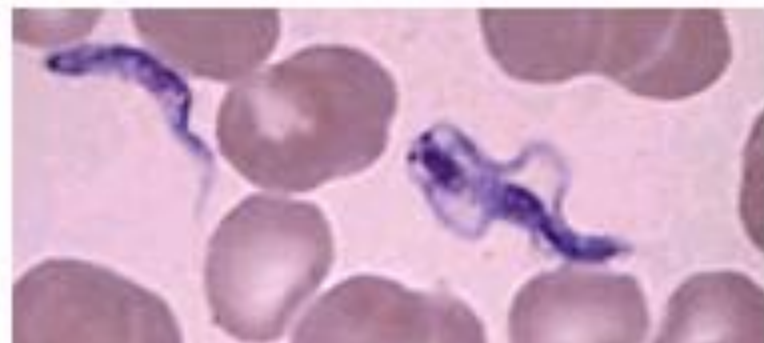
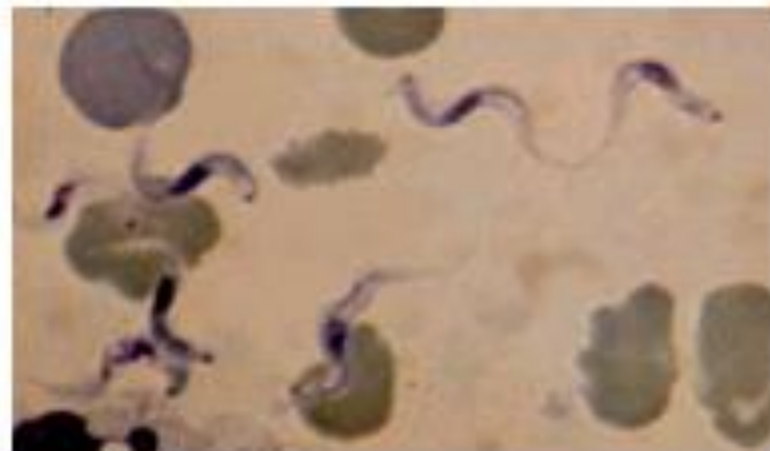
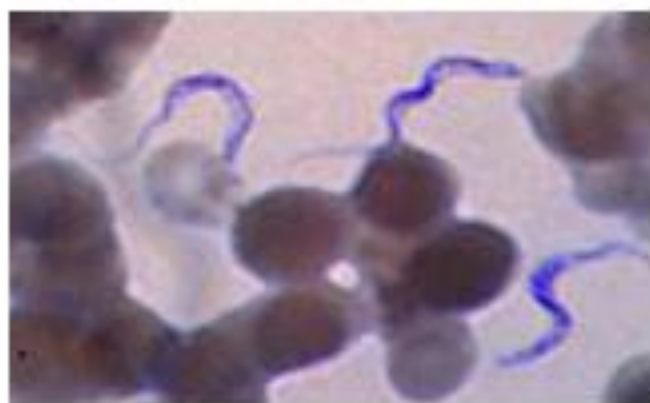


Fig : Trypanosoma gambiense or rhodesiense Trypomastigote stage,
Blood smear (X 1000)
the Trypomastigote stage also found in lymph node juice, C. S. F. ,
bone marrow biopsy



Tsetse fly

Early infection



Skin after the bite of Tse
– tse fly



Early infection



Late infection

2- *Trypanosoma cruzi*

Diseases: American trypanosomiasis or chaga's disease.

Mode of infection

- 1-Bite of infected insect (reduviid bug)
- 2-blood transfusion
- 3-organ transplantation
- 4- Transplacental
- 5-Sexual intercourse
- 6-Through contaminated syringes and needles

life cycle of *Trypanosoma cruzi*. •

Triatomine Bug Stages

Human Stages

- 1** Triatomine bug takes a blood meal (passes metacyclic trypomastigotes in feces, trypomastigotes enter bite wound or mucosal membranes, such as the conjunctiva)

- 2** Metacyclic trypomastigotes penetrate various cells at bite wound site. Inside cells they transform into amastigotes.

- 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.

- 4** Intracellular amastigotes transform into trypomastigotes, then burst out of the cell and enter the bloodstream.

- 5** Triatomine bug takes a blood meal (trypomastigotes ingested)

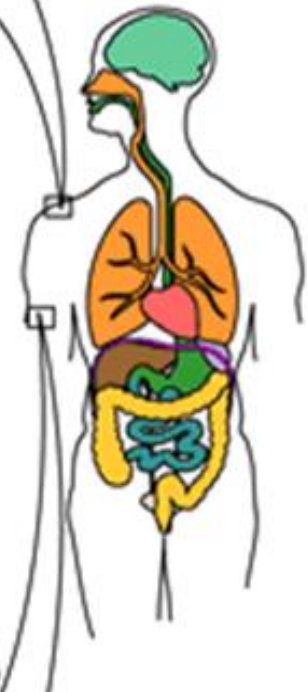
i = Infective Stage
d = Diagnostic Stage

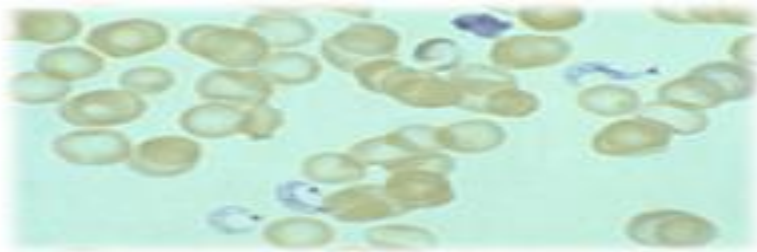
- 6** Epimastigote stage in midgut

Multiplies in midgut

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Metacyclic trypomastigotes in hindgut



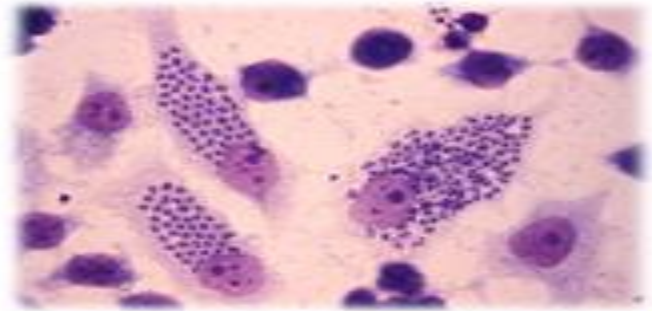


C-shape Trypomastigote, blood smear



Triatoma infestans

Reduviid bug, vector(chagas disease)



Amastigote from biopsy of cardiac muscle



Chagas disease

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Diagnosis of cutaneous&mucocutaneous leishmaniasis

- Smear: Giemsa stain – microscopy for LD bodies (amastigotes)
- Leishman skin test(montenegro test)
- Biopsy: microscopy for LD bodies or culture in Schneiders Drosophila medium, NNN medium (Novy-Macneal-Nicolle).the material from patient (splenic aspirate, bone marrow, liver aspirate or blood) parasites grow as promastigotes
- Immunological testing
- PCR

Leishmanian or Montenegro test:

- * An intradermal test for delayed hypersensitivity
- * Most reliable for cutaneous leishmaniasis.
- * A suspension of 10^7 /ml promastigotes in 0.5% phenol/saline intradermally.
- * Test is read after 2-3 days
- . **Montenegro test (leishmania test):** erythema and induration occur on skin within 48hours.



Visceral leishmaniasis Diagnosis

1) direct method

Parasitological diagnosis:

METHOD

Bone marrow aspirate

Splenic aspirate

Lymph node

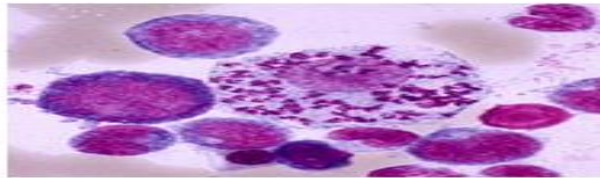
Tissue biopsy

1. microscopy

2. culture in NNN medium

3. thick film method

Bone marrow aspiration



Bone marrow amastigotes

2) Indirect method

- Immunological Diagnosis
- Specific serologic tests: Direct Agglutination Test (DAT), ELISA, IFAT
- Skin test (leishmanin test)(montenegro test) for survey of populations and follow-up after treatment.
- Blood count PCR

Laboratory Diagnosis of African trypanosomiasis

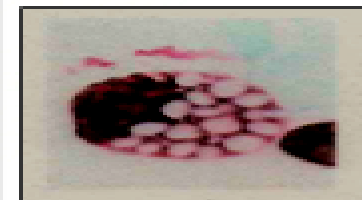
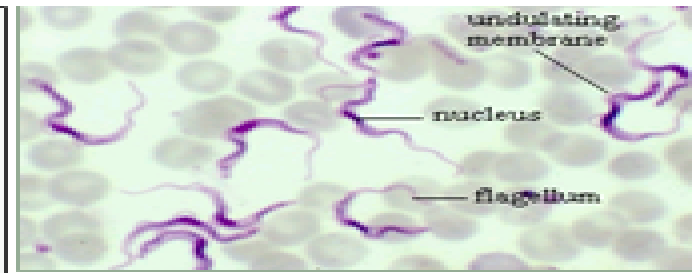
Direct Laboratory Diagnosis

To demonstrate the parasite

Early: in chancre aspirate, blood, lymph node, bone marrow &

Late: in CSF BY :-

- Microscopic examination of fresh unstained or Giemsa stained films: >>> polymorphic trypomastigote.
- Culture (NNN or Weinmann's media: >>> epimastigote.
- Animal inoculation
- CSF examination: Trypomastigotes, and Morula cells; vacuolated plasma cell.



Morula cell of Mott



Aspiration of swollen gland



Lumbar puncture for CSF

By Detection of specific **antigens** in the blood :-

- **Card Agglutination Trypanosomiasis Test [CATT]**: It is a simple & rapid test for detection of **circulating antigens** in the blood of the patient. It is useful in surveys specially for *T. b. gambiense*.
- Molecular techniques (e.g. PCR)

Indirect Laboratory diagnosis

by Detecting anti-Trypanosoma Abs by serological methods (ELISA, IFA, IHA etc..), But can't distinguish between current and previous infections .

Laboratory Diagnosis : by demonstration of *T.cruzi* in blood or tissues, or by serology .

1. Microscopic demonstration of parasite
from stained blood smear or
lymph node aspirate .



Guinea pig inoculation may be done with blood, CSF, lymph node aspirate or other tissue materials and the trypanomastigote looked for in its blood smears.

2. **Blood culture** : using NNN medium

3. **Serological tests** :-

- detection of antibodies include complement fixation test, indirect haemagglutination, immunofluorescence and ELISA.
- demonstration of the parasite antigen in blood and urine.

4. **Xenodiagnosis**

may be attempted by allowing a parasite free reduviid bug to bite the patient and by demonstrating the parasite in its intestinal contents.



5. **intradermal test** : described for demonstration of hypersensitivity.