



**Medical Analysis
Department**

Trypanosoma sp. & Leishmania sp.

Parasitology (MA 301)

Summer School

Lab 5

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Stages of Family Trypanosomatidae

Hemoflagellates pass through four morphological stages:

- Amastigote
- Promastigote
- Epimastigote
- Trypomastigote

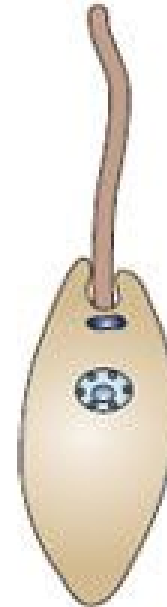
Morphology of family: Trypanosomatidae



Trypomastigote



Amastigote



Promastigote



Epimastigote

Trypanosoma brucei

Introduction

- The blood and tissue flagellates belong to the family *Trypanosomatidae*.
- **African Trypanosomiasis** is also known as **African Sleeping sickness** a general term used to refer to human diseases caused by hemoflagellates of the genus *Trypanosoma*. These diseases have been well documented through the ages.



Habitat

Trypanosomes infect humans and other vertebrates. They primarily multiply in connective tissues, then spread to lymph nodes, blood, and eventually the central nervous system.

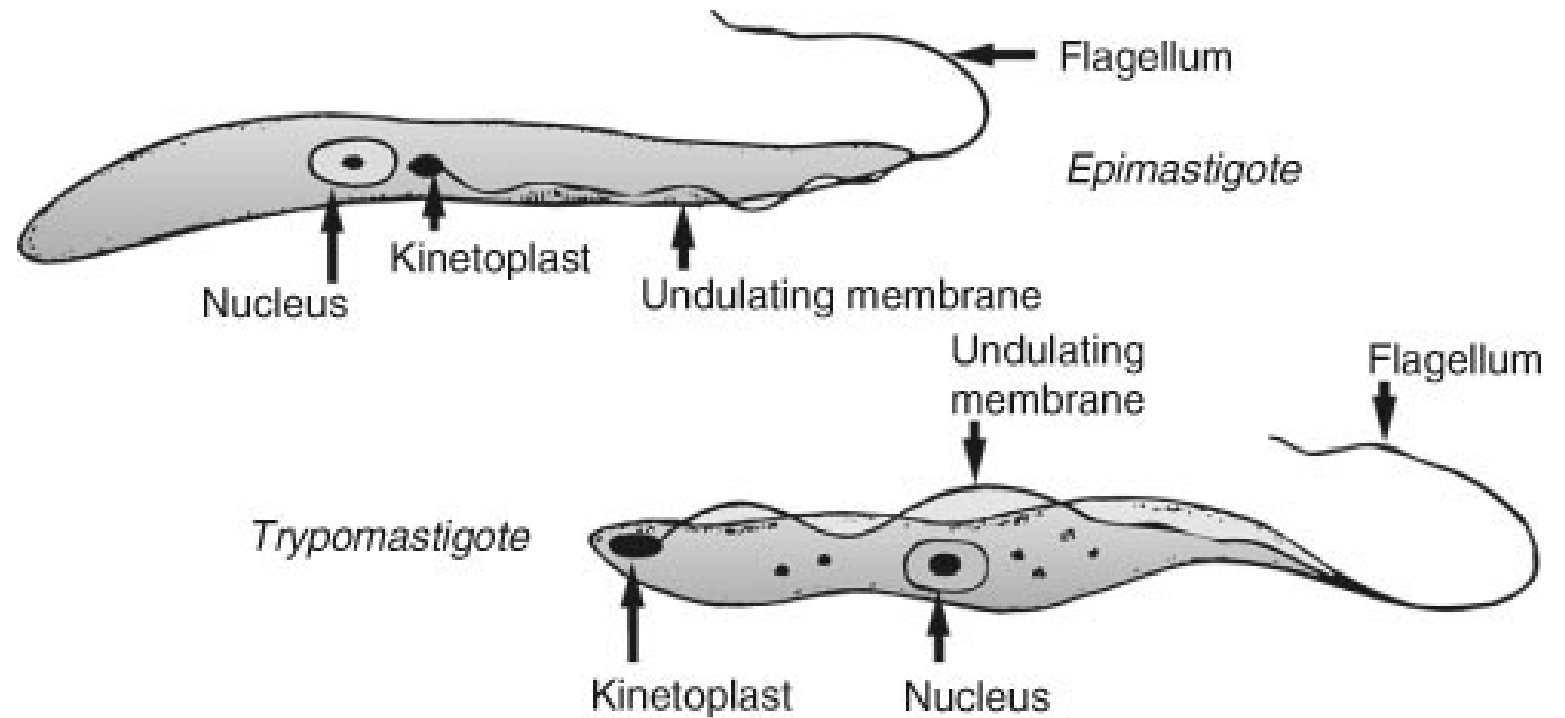
Morphology

- In vertebrate hosts, *T. brucei* appears as pleomorphic **trypomastigotes**: long slender, short stumpy, or intermediate forms
- In fresh blood, they are motile, spindle-shaped, and spin around RBCs.
- In insect vectors, they occur as **epimastigote** and **metacyclic trypomastigote** forms.

Stages of *Trypanosoma Brucei*

1- Epimastigote Stage:

2- Trypomastigote Stage:



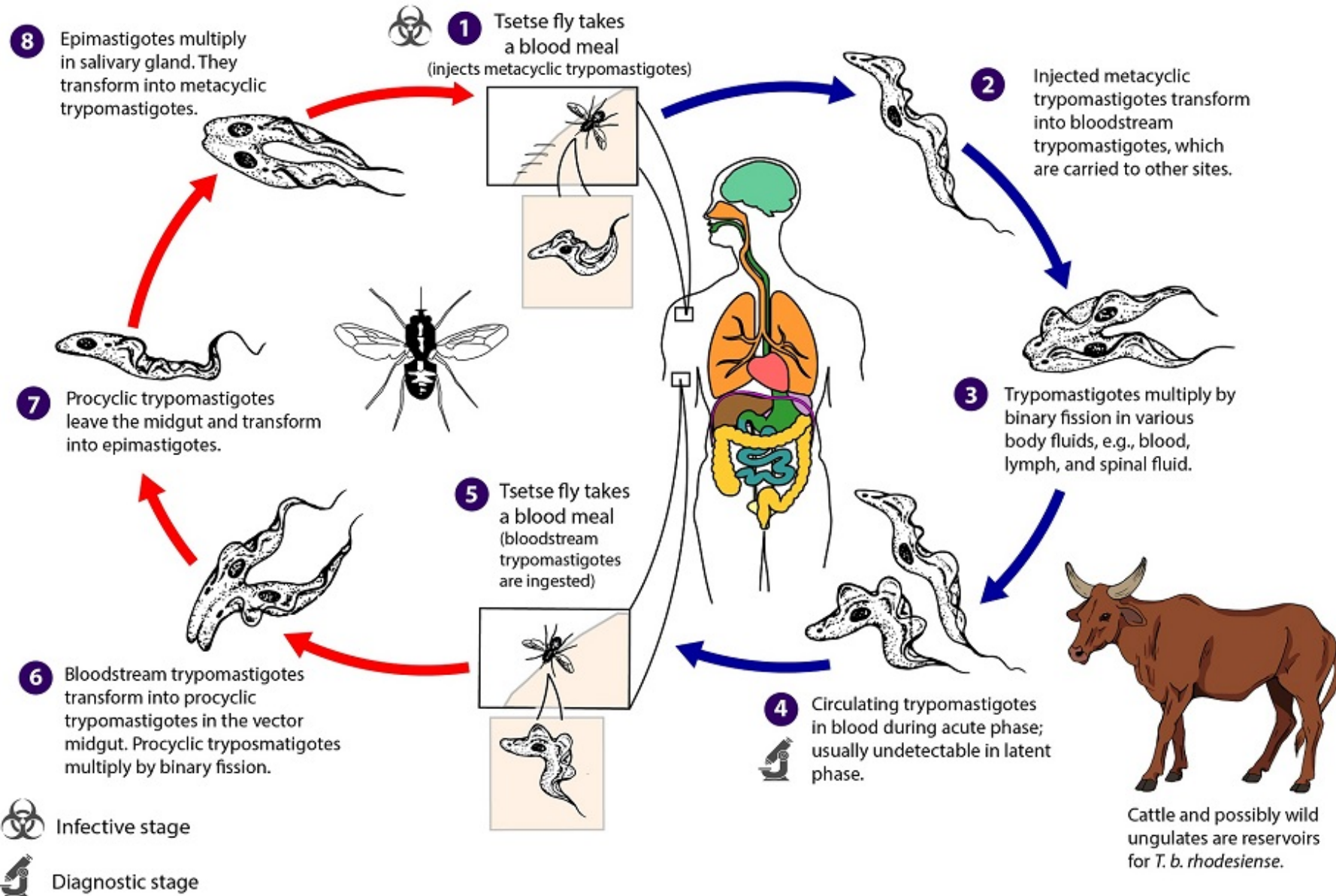
Vector

- *Glossina* sp. (Tsetse Fly)



Tsetse Fly Stages

Mammalian Stages



Sample do we take for Trypanosomiasis

- 1- Blood
- 2- Lymph Aspiration
- 3- Cerebrospinal fluid



Laboratory Diagnosis

1- Microscopy

Wet mounts of lymph node aspirates, chancre fluid, and CSF can rapidly show motile parasites.

Giemsa-stained smears (thick or concentrated blood films) detect trypomastigotes; concentration methods increase sensitivity when parasitemia is low.

Laboratory Diagnosis

2- Serodiagnosis:

Antibody detection: Almost all patients with African trypanosomiasis have very high levels of total serum IgM antibodies and later, CSF IgM antibodies. Various serological methods have been developed to detect these antibodies, and are as follows:

- Indirect hemagglutination (IHA)
- Enzyme-linked immunosorbent assay (ELISA)

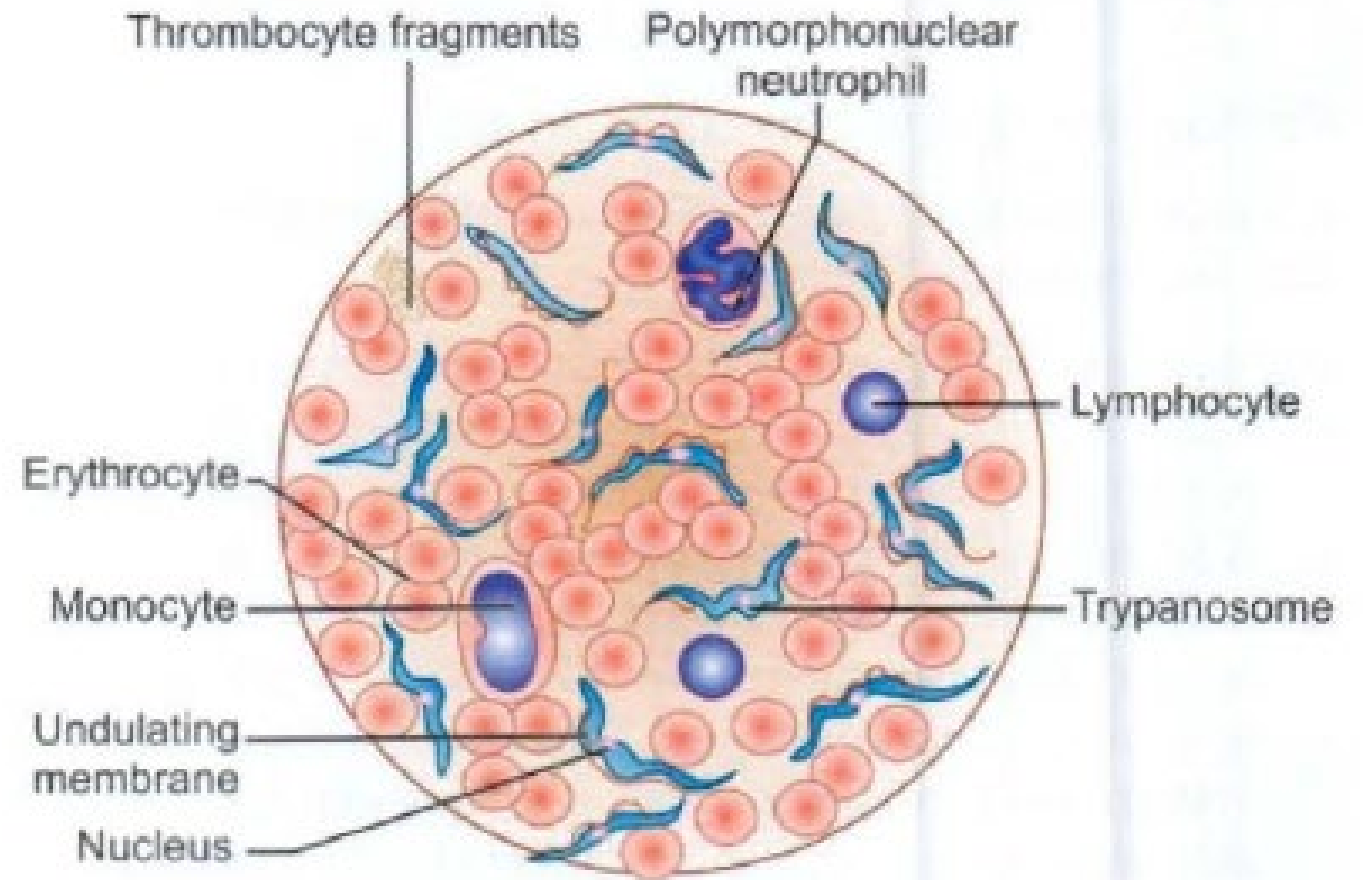
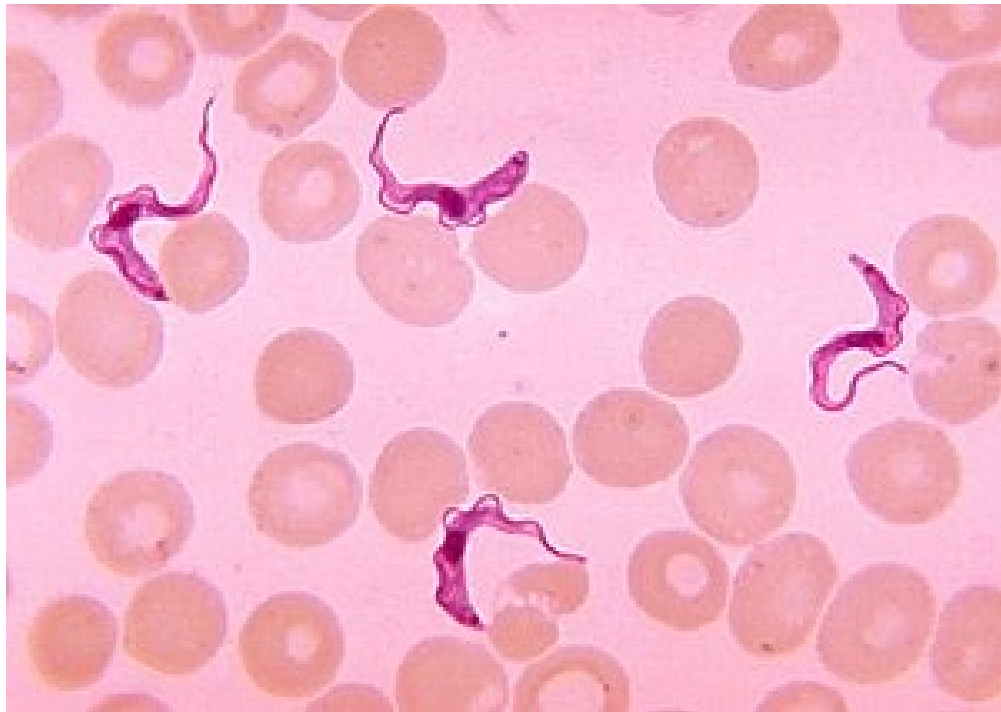
Note: Specific antibodies are detected by these tests in serum within 2-3 weeks of infection. Specific antibodies in CSF are demonstrated by ELISA

Laboratory Diagnosis



Others

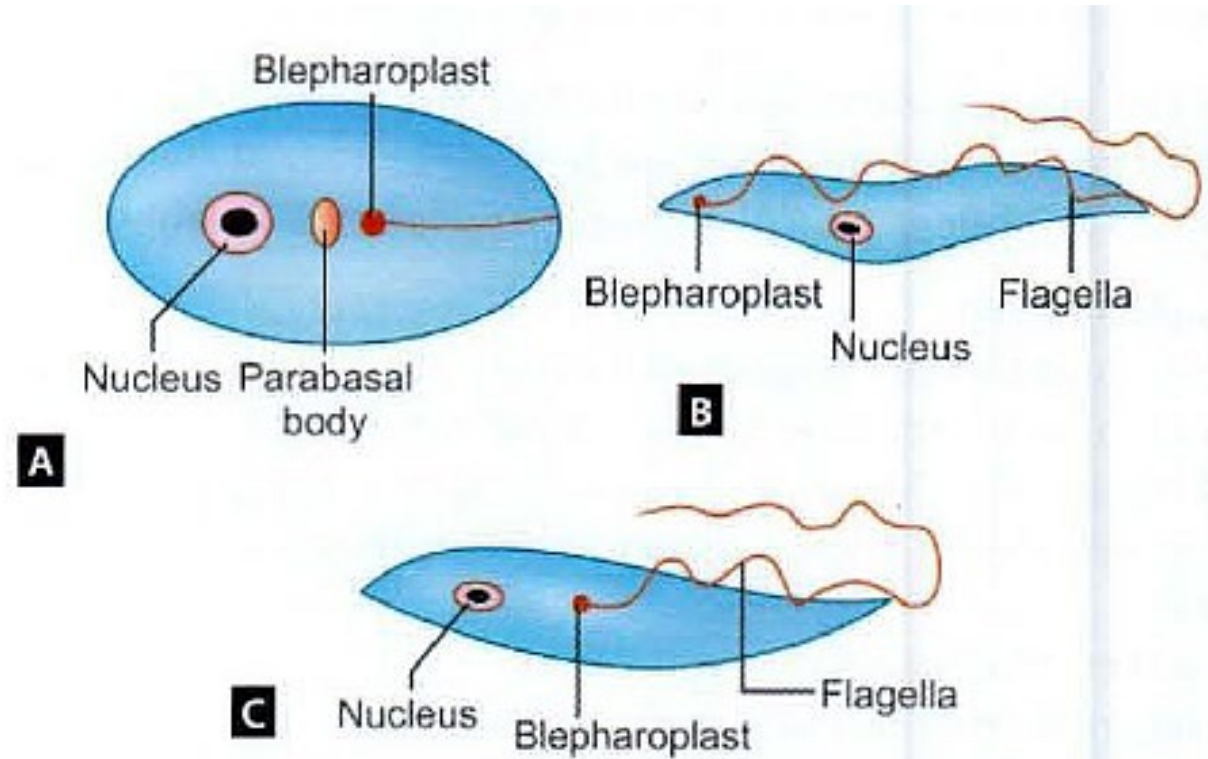
3. **Antigen detection:** Antigens from serum and CSF can be detected by ELISA.
4. **Imaging:** Computed tomography (CT) scan of the brain shows cerebral edema, and magnetic resonance imaging (MRI) shows white matter enhancement in patients with late-stage central nervous system involvement



Trypanosoma cruzi

Stages of *Trypanosoma cruzi*

- 1- Amastigote
- 2- Trypomastigote
- 3- Epimastigote



Figs 5A to C: *Trypanosoma cruzi*. (A) Amastigote; (B) Trypomastigote; and (C) Epimastigote

Habitat

- **Amastigotes** are the intracellular parasites. They are found in muscular tissue, nervous tissue and reticuloendothelial system.
- **Trypomastigotes** are found in the peripheral blood.
- In Triatomine bugs, **Epimastigote** forms are found in the midgut, and metacyclic **trypomastigote** forms are present in hindgut and feces.

Morphology

Amastigote:

- Amastigotes are oval bodies measuring 2-4 μm in diameter having a nucleus and kinetoplast.
- Flagellum is absent. Morphologically, it resembles the amastigote of *Leishmania* spp., hence, it is frequently called as *leishmanial form*.
- Multiplication of the parasite occurs in this stage.
- This form is found in muscles, nerve cells, and reticuloendothelial systems.

Morphology

Trypomastigote:

- Trypomastigotes are non-multiplying forms found in the peripheral blood of man and other mammalian hosts.
- In the blood, they appear either as long, thin flagellates about (20 mcm long) or short, stumpy form (15 μm long).
- In stained blood smears, they are shaped like alphabet "C"; "U"; or "S"; having a free flagellum of about one-third the length of the body.
- These forms do not multiply in humans and are taken up by the insect vectors.

Morphology

Epimastigote form:

- Epimastigote forms are found in the insect vector, the reduviid bug, and in culture also.
- It has a kinetoplast adjacent to the nucleus.
- An undulating membrane runs along the anterior half of the parasite.
- Epimastigotes divide by binary fission in the hindgut of the vector.

Vector

Triatoma infestans (Kissing bug)

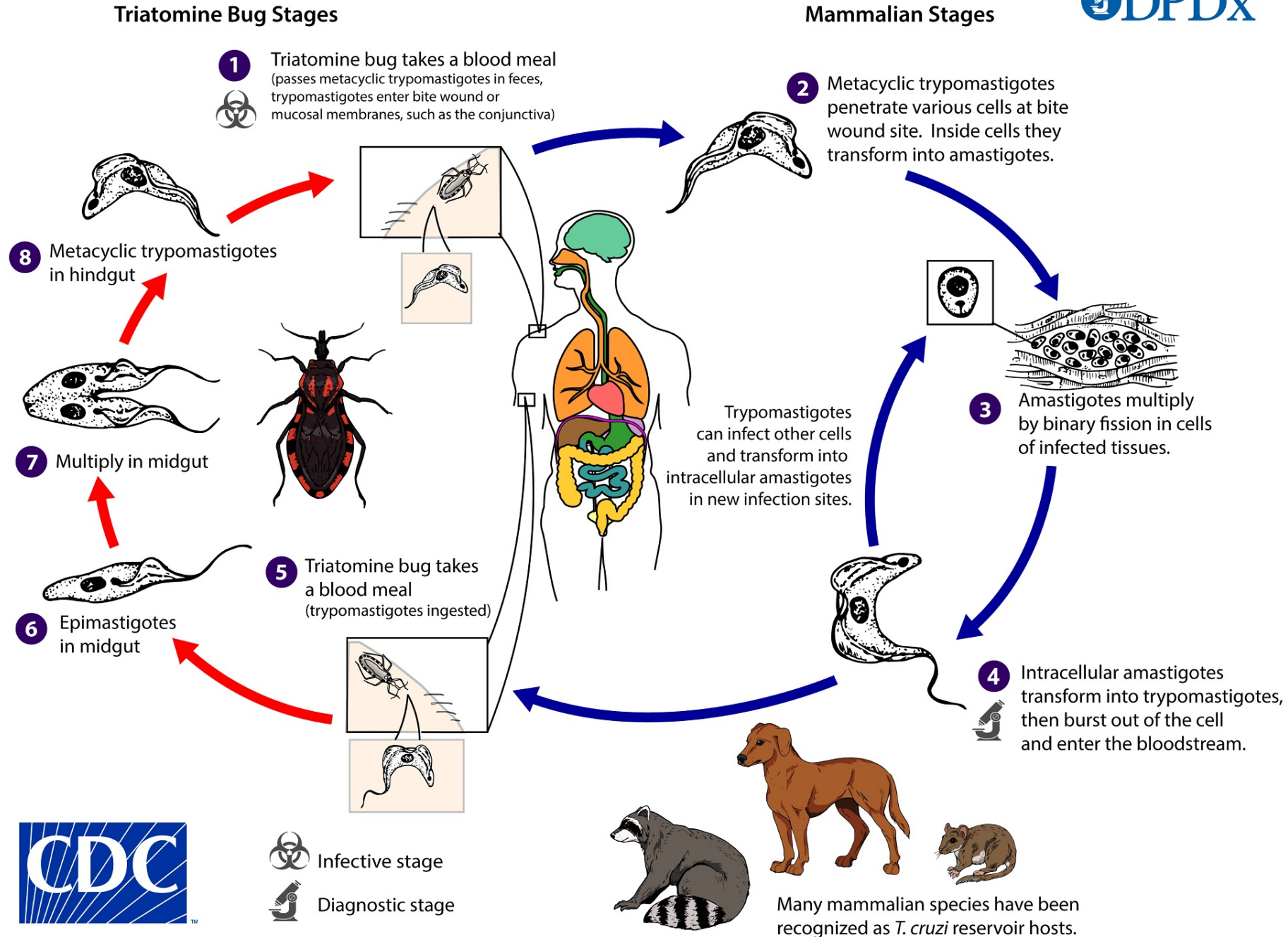


Life cycle



- Infection begins when triatomine bugs (kissing bugs) feed on human blood and release infective metacyclic trypomastigotes in their feces near the bite site.
- The parasites enter the body through mucous membranes or breaks in the skin.
- Inside host cells, trypomastigotes transform into amastigotes, which multiply by binary fission.
- Amastigotes differentiate back into trypomastigotes, which burst out of cells and spread via the bloodstream to infect new tissues.
- Triatomine bugs ingest bloodstream trypomastigotes during a blood meal.
- In the insect midgut, parasites become epimastigotes, multiply, and then transform into infective metacyclic trypomastigotes in the hindgut.
- The cycle repeats when the infected bug bites another host.

Trypanosoma cruzi



Samples we take for *Trypanosoma cruzi*

Acute phase:

- Blood → for detecting motile trypomastigotes by microscopy.

Chronic phase:

- Serum or plasma → for detecting anti-T. cruzi antibodies (ELISA, IFA).
- Blood → for PCR to detect parasite DNA.
- Tissue biopsy (heart, esophagus, colon) in some cases to identify amastigotes in lesions

Diagnosis

Diagnosis is made by demonstrating *T. cruzi* in blood or tissues, or by serology.

1- Microscopy:

- The diagnosis of acute Chagas disease requires the detection of parasites.
- In a wet mount, trypomastigotes are faintly visible, but their snake-like motion against red blood cells (RBCs) makes their presence apparent.
- Trypomastigotes can also be seen in thick and thin peripheral blood smear, stained with Giemsa stain

Note: Serologic testing plays no role in diagnosing acute Chagas disease.

Diagnosis



2- Serology:

Antigen detection: T. cruzi antigen can be detected in urine and serum in patients with chronic Chagas disease. ELISA has been developed for the detection of antigens.

Antibody detection: Antibodies (IgG) against T. cruzi may be detected by ELISA

Diagnosis



3- Xenodiagnoses:

This is the method of choice in suspected Chagas disease, if other examinations are negative, especially during the early phase of the disease onset.

Principle

A laboratory-clean, uninfected triatomine bug (reduviid bug) is allowed to feed on the patient's blood. If *Trypanosoma cruzi* is present in the patient—even in very low numbers—the parasite will be ingested by the bug, where it can multiply in the insect's gut and become detectable later.

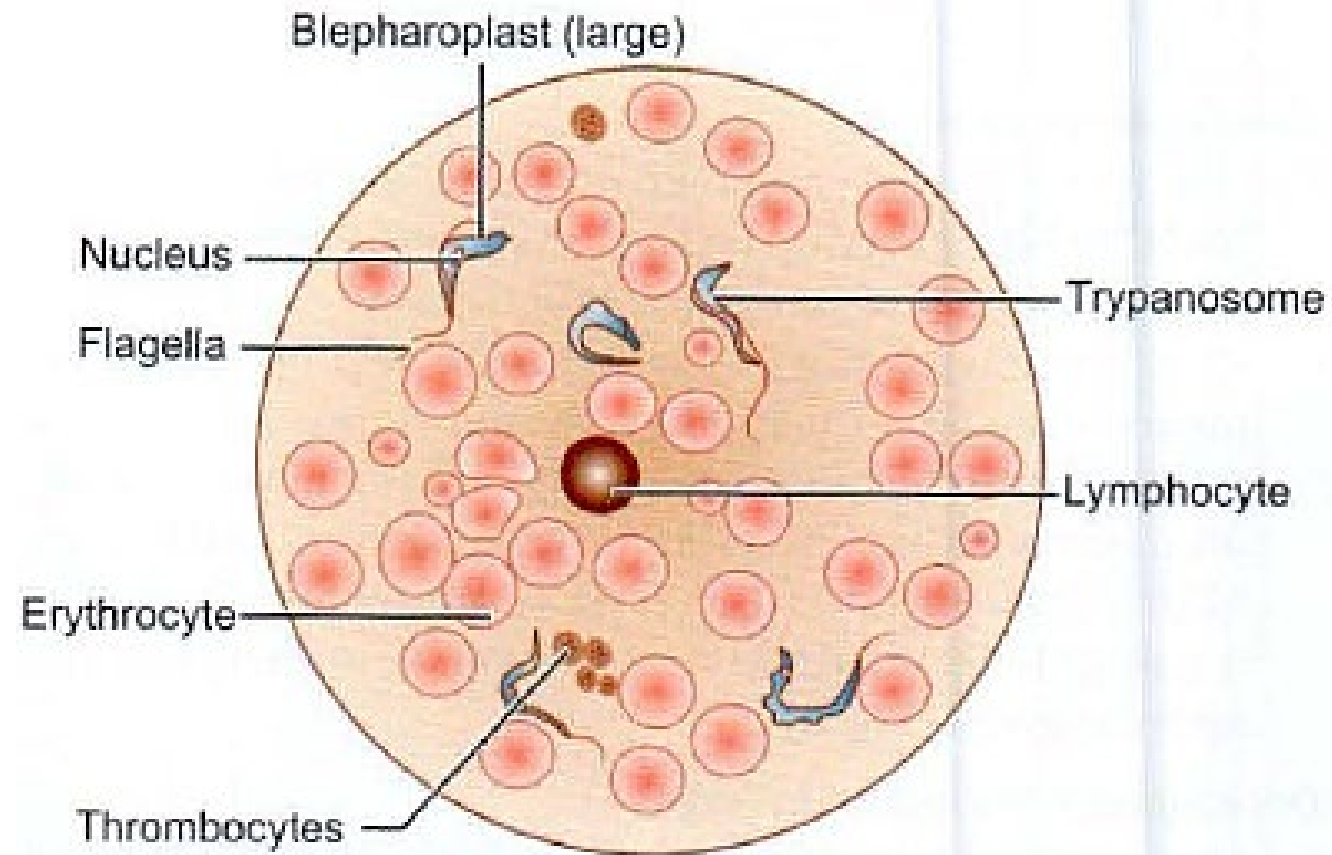
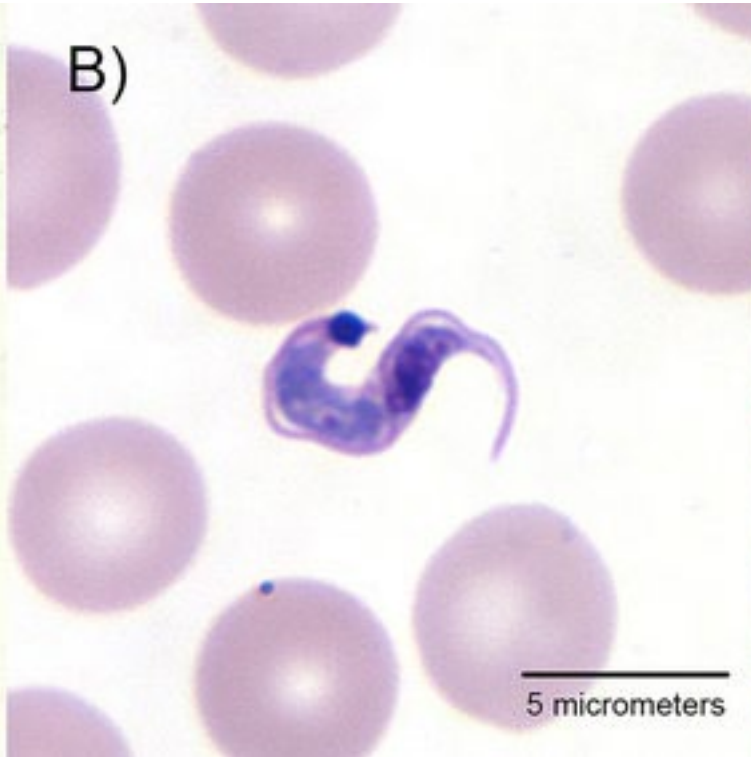


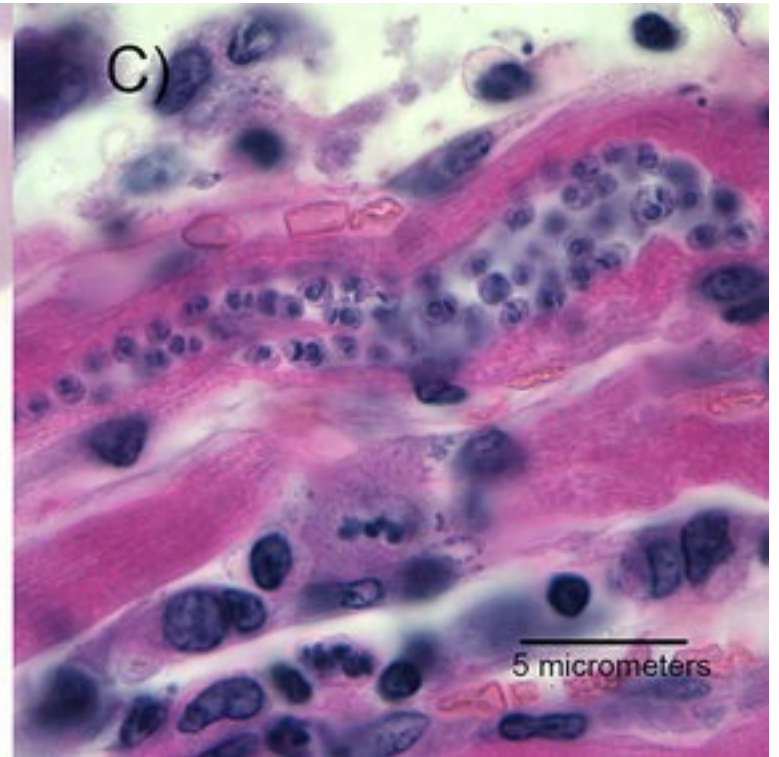
Fig. 7: *Trypanosoma cruzi*, blood smear Giemsa stain, magnification 1100X



Epimastigote



Trypomastigote



Amastigote

***Leishmania* sp.**

Introduction

Leishmania are obligate intracellular parasites with two hosts: mammals and female sandflies.

- In mammals, found inside macrophages as **amastigotes** (oval, with nucleus and kinetoplast).
- In sandflies, they exist as **promastigotes** (spindle-shaped, with a single anterior flagellum).

Types of *Leishmania* sp.

Across the tropics, three different diseases are caused by various species of the genus *Leishmania*. These are:

1. ***Visceral leishmaniasis***: The species *Leishmania donovani* complex infecting internal organs (liver, spleen and bone marrow) of humans are the causative parasite.
2. ***Cutaneous leishmaniasis***: The species *Leishmania tropica* is the causative parasite.
3. ***Mucocutaneous leishmaniasis***: It is caused by the *Leishmania braziliensis* complex.

Morphology

Amastigote:

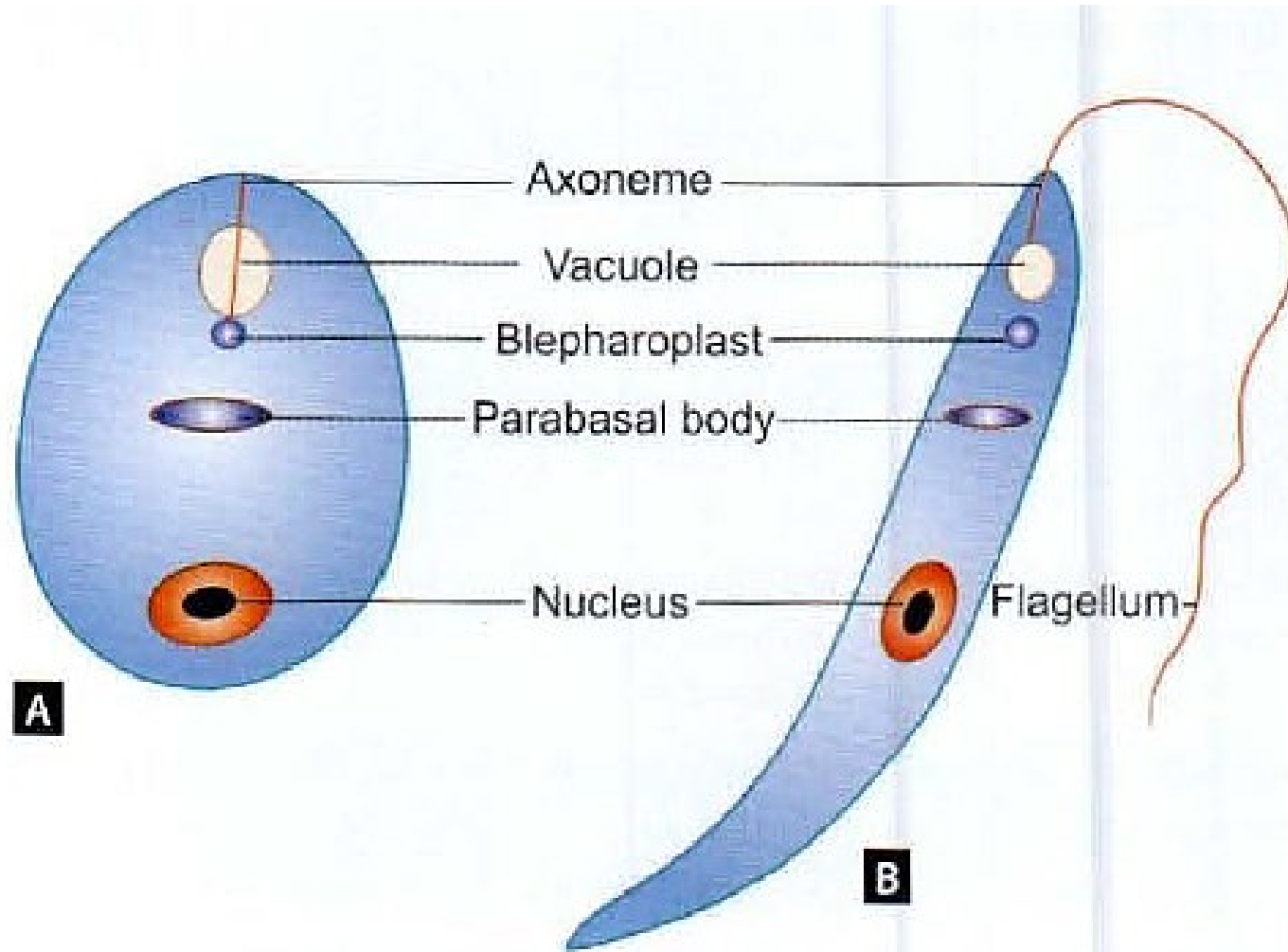
The amastigote form is an ovoid or rounded cell, about 2-4 μm in size.

- It is typically intracellular, being found inside macrophages, monocytes, neutrophils, or endothelial cells.

Morphology

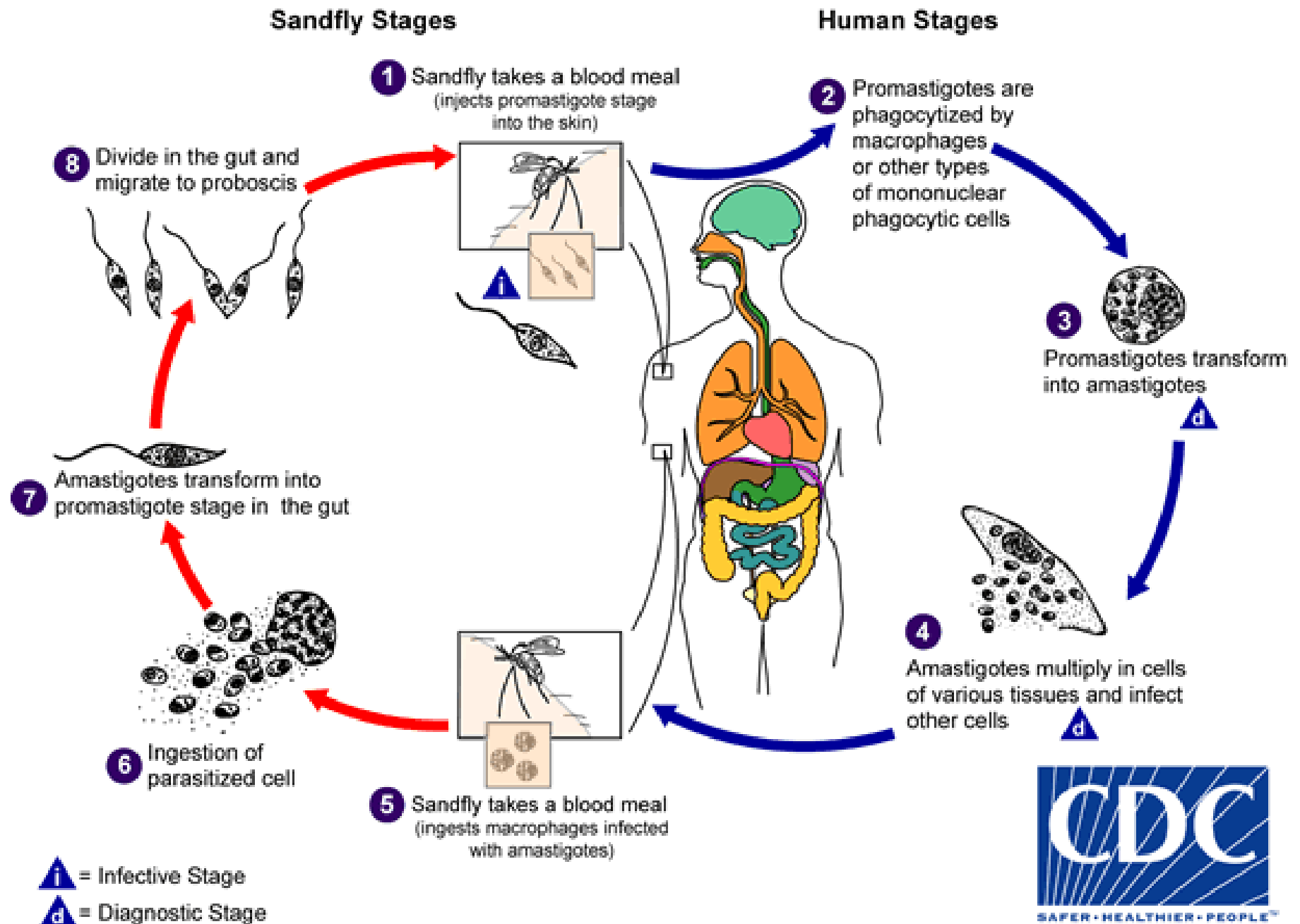
Promastigote:

- It is a flagellar stage and is present in the insect vector, sandfly, and in cultures.
- The promastigotes, which are initially short, oval, or pear-shaped forms, subsequently become long, spindle-shaped cells, 15- 25 μm in length and 1.5-3.5 μm in breadth.
- A single nucleus is situated at the center.
- The flagellum is single, delicate, and measures 15-28 μm .



Figs 9A and B: Morphology of *Leishmania donovani*. (A) Amastigote [Leishman-Donovan (LD) body]; and (B) Promastigote

Life cycle



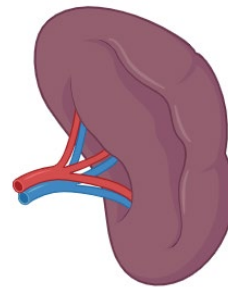
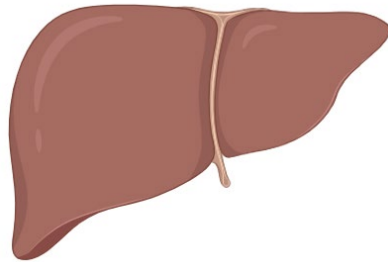
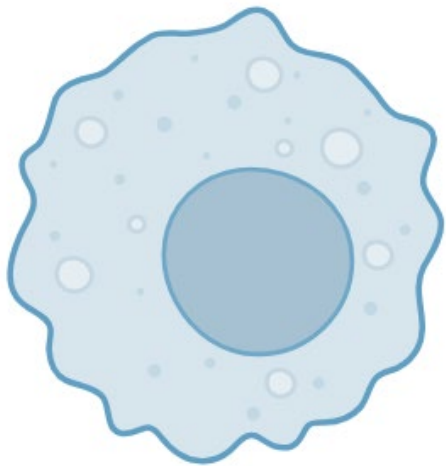
Leishmania donovani
(Old World Leishmaniasis)

Introduction

Leishmania donovani causes Visceral Leishmaniasis or ***kala-azar***.

Habitat

The amastigote of *Leishmania donovani* is found in the reticuloendothelial system. They are found mostly within the **macrophages** in the spleen, liver, bone marrow, and less often in other locations such as skin, intestinal mucosa, and mesenteric lymph nodes.



Life cycle

Leishmania donovani completes its life cycle in two hosts

Host: Man, dog, and other mammals.

Vector: Female sandfly (*Phlebotomus* species).

Infective form: Promastigote form present in the midgut of the *female* sandfly.

Vector

Sandfly (*Phlebotomus* sp.)



Diagnosis

1. Microscopical:

Demonstration of amastigotes in tissue smears (spleen, bone marrow, lymph node).



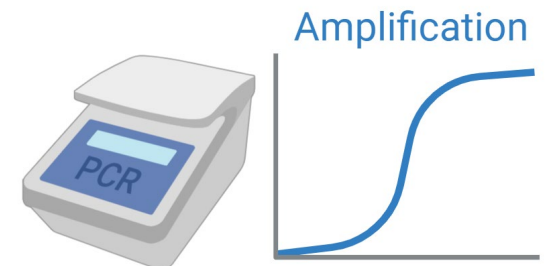
2. Serological:

rk39 rapid diagnostic test, ELISA, IFAT.

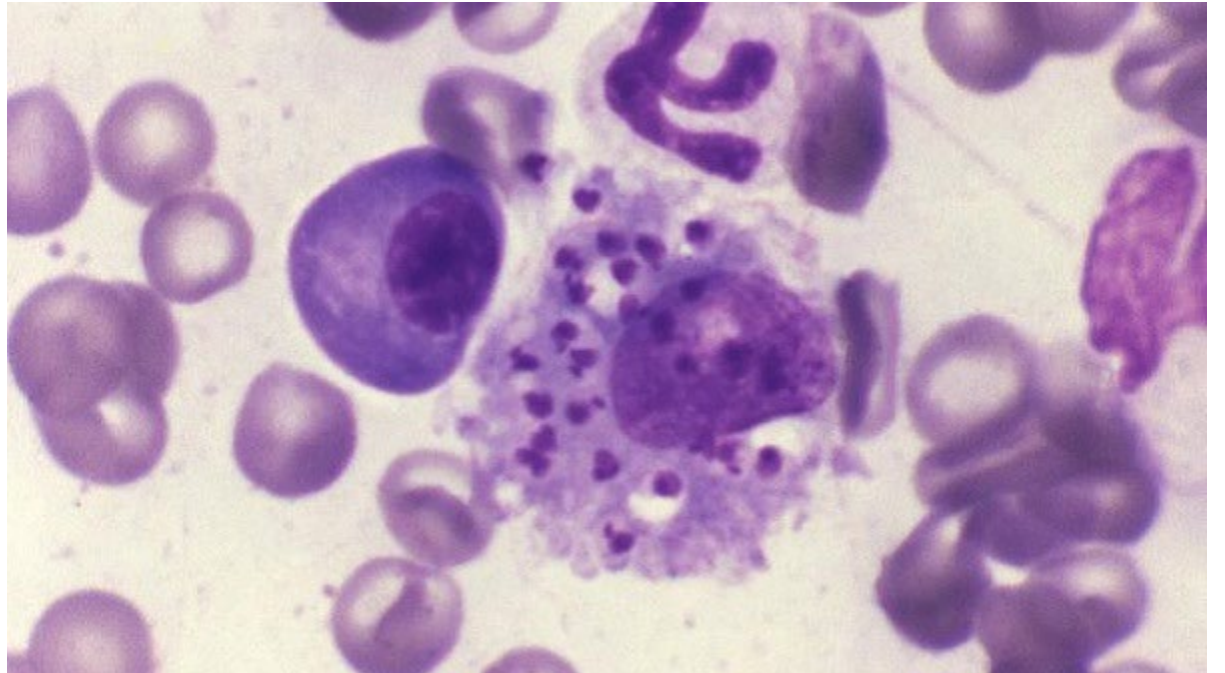
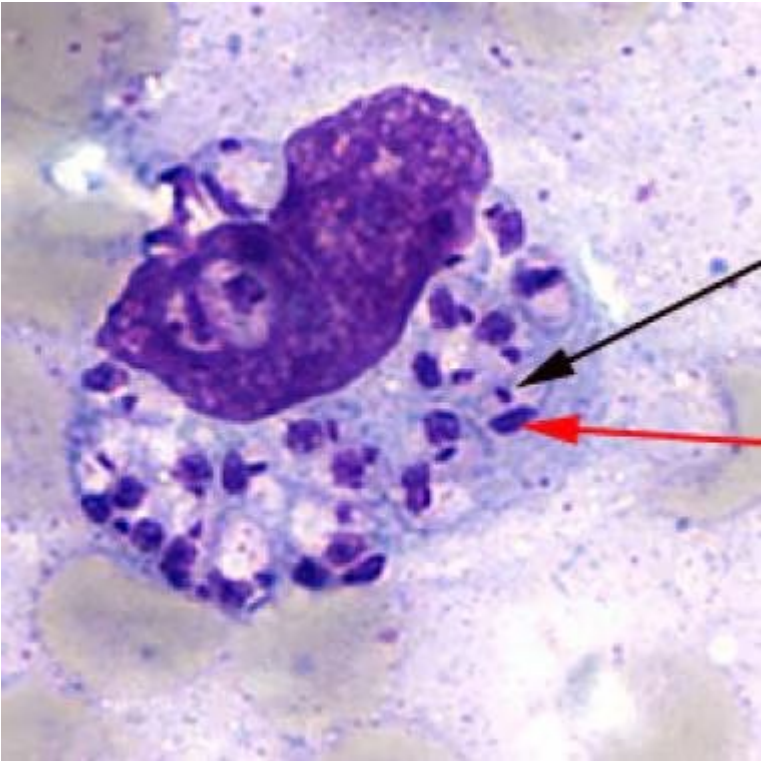


3. Molecular:

PCR (high sensitivity, confirmatory).



Leishmania sp. In blood smear



Leishmania tropica
(cutaneous Leishmaniasis)

Introduction

causes old-world cutaneous leishmaniasis. The disease is also known as *oriental sore*, *Delhi boil*, *Bagdad boil*, or *Aleppo button*.

Distribution

L. tropica is found in the Middle East, India, Afghanistan, Eastern Mediterranean countries, and North Africa.

Habitat

L. tropica, causing CL (old world CL) is essentially a parasite of skin. The amastigote forms occur in the reticuloendothelial cells of the skin, whereas promastigote forms are seen in sand fly vector.

Life cycle

Leishmania trpoica completes its life cycle in two hosts

Host: Man, dog, and other mammals.

Vector: Female sandfly (*Phlebotomus* species).

Infective form: Promastigote form present in the midgut of the *female* sandfly.

Vector

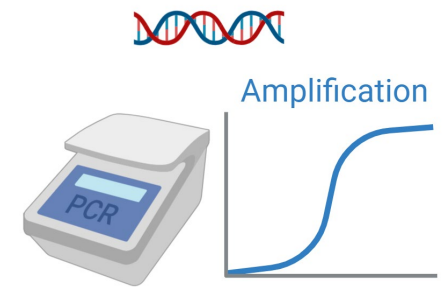
Sandfly (*Phlebotomus* sp.)



Diagnosis

Diagnosis of *Leishmania tropica* (Cutaneous leishmaniasis):

- **Microscopical:** Demonstration of **amastigotes** in skin smear or biopsy.
- **Molecular:** PCR for species confirmation.
- **Serology:** Limited value (since disease is localized).



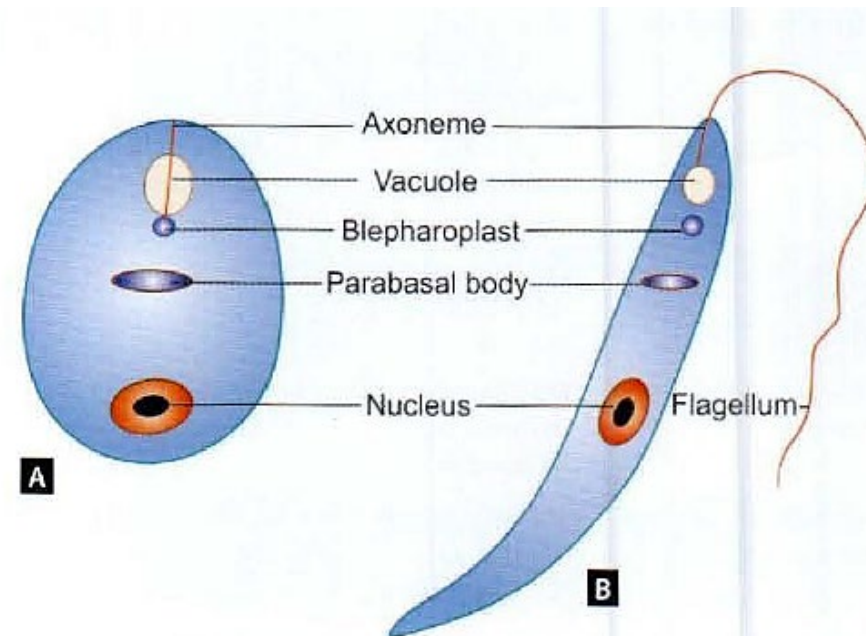
Leishmania Braziliensis
(New World Leishmaniasis)

Habitat

These occur as intracellular parasite. The amastigote form is seen inside the macrophages of skin and mucous membrane of the nose and buccal cavity. The promastigote form occurs in vector species *Lutzomyia*.

Morphology

Morphology of amastigote and promastigote forms of both the parasites is same as that of the other two species of *Leishmania*.



Figs 9A and B: Morphology of *Leishmania donovani*. (A) Amastigote [Leishman-Donovan (LD) body]; and (B) Promastigote

Vector

Sandfly (*Lutzomyia* sp.)



Life cycle

Leishmania braziliensis completes its life cycle in two hosts

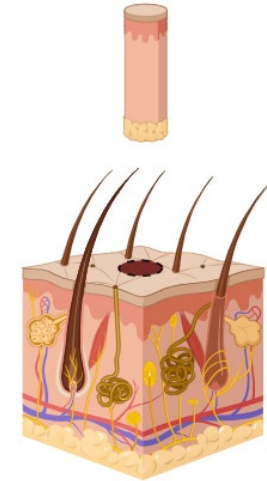
Host: Man, dog, and other mammals.

Vector: Female sandfly (*Lutzomyia* species).

Infective form: Promastigote form present in the midgut of the *female* sandfly.

Diagnosis

- **Microscopy:** Amastigotes are demonstrated in smears taken from lesions of skin and mucous membrane.
- **Biopsy:** Amastigotes can also be demonstrated from slit-skin biopsy.
- **Serology:** ELISA is a sensitive method to detect antibody; being positive in 85% of cases.



References

- Paniker, C. K. J. & Ghosh, S. 2021. *Paniker's textbook of medical parasitology*, New Delhi, Jaypee Brothers Medical Publishers.