Tissue Parasites Protozoans: Hemoflagellates

Leishmania and *Trypanosoma*

Definitions

- **amastigote.** A small, ovoid, nonflagellated form found in the life cycle of *Trypanosoma*. Notable structures include a mitochondrial kinetoplastid and a large nucleus. Also called L.D. (Leishman-Donovan) body or leishmanial form.
- **arthropod.** An organism having a hard, segmented exoskeleton and paired, jointed legs
- **blepharoplast.** The basal body origin of the flagella that supports the undulating membrane in kinetoplastid flagellates.
- **cutaneous.** Pertaining to the skin.

Definitions

- **diffuse (disseminated) cutaneous leishmaniasis (DCL).** A chronic form of leishmaniasis caused by *Leishmania aethiopia* in Ethiopia and Kenya and by various subspecies of *L. mexicana* in Central and South America, and characterized by non-ulcerating, non-necrotizing skin lesions that spread over the body.
- **epimastigote.** Flattened, spindle-shaped, flagellated form seen primarily in the gut (e.g., in the reduviid bug) or salivary glands (e.g., in the tsetse fly) of the insect vectors in the life cycle of trypanosomes; it has an undulating membrane that extends from the flagellum (attached along the anterior half of the organism) to the small kinetoplast located just anteriorly to the larger nucleus located at the midpoint of the organism.
- **kinetoplast.** An accessory body found in many protozoa, especially in the family Trypanosomatidae, consisting of a large mitochondrion next to the basal granule **(blepharoplast)** of the anterior or undulating membrane flagellum; contains mitochondrial DNA.

Definitions

- **L.D. body (Leishman-Donovan body).** Each of the small ovoid **amastigote** forms found in tissue macrophages of the liver and spleen in patients with *Leishmania donovani* infection.
- **promastigote.** A flagellate form of *Trypanosoma* in which the kinetoplast is located at the anterior end of the organism; it has no undulating membrane. This form is seen in the midgut and pharynx of sandfly vectors in the life cycle of the leishmania parasites and is the form that develops in culture media in vitro.
- **trypomastigote.** A flagellate form with the **kinetoplast** located at the posterior end of the organism and the undulating membrane extending along the entire body from the flagellum (anterior end) to the posterior end at the blepharoplast. This form is seen in the blood of humans with trypanosomiasis and is the infective stage transmitted by the insect vectors.

Definitions

- **subpatent.** Not evident, subclinical.
- **Winterbottom's sign.** Seen in the early phase of African trypanosomiasis, exhibiting swelling of the cervical chain of lymph nodes along the back of the neck when trypanosomes travel in the lymphatic fluid causing inflammation.
- **xenodiagnosis.** Infections with *Trypanosoma cruzi* may be diagnosed by allowing an uninfected Triatoma bug to feed on the patient (the bite is painless); the insect's feces are later examined for parasites (trypomastigote forms).

Hemoflagellates

Scientific Name	Common Name
Trypanosoma brucei rhodesiense (trip-an”o-so”muh/brew’see”l/ro-dee”zee-en’see)	East African sleeping sickness
Trypanosoma brucei gambiense (trip-an”o-so’muh/brew’see”l/gam-bee-en’see)	West African sleeping sickness
Trypanosoma cruzi (trip-an”o-so’muh/kroo’zye)	Chagas disease
Leishmania tropica complex (leesh-may’nee-uh/trop’i-kuh)	Oriental boil
Leishmania mexicana complex (leesh-may’nee-uh/mex-l-can’uh)	New World leishmaniasis, diffuse cutaneous leishmaniasis
Leishmania braziliensis complex (leesh-may’nee-uh/bra-zil”l-en’sis)	New World leishmaniasis
Leishmania donovani complex (leesh-may’nee-uh/don”o-vay’nigh)	Kala-azar

Classification of Blood and Tissue Protozoa: Hemoflagellates:

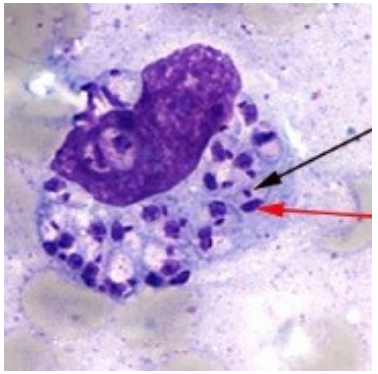
Hemoflagellates:

Leishmaniasis

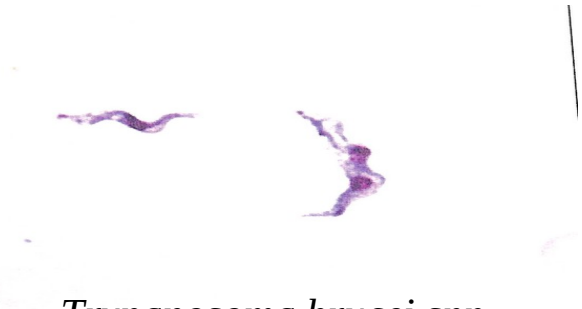
- ***Leishmania donovani***
- ***Leishmania tropica***
- ***Leishmania braziliensis***

Trypanosomiasis

- ***Trypanosoma brucei gambiense***
- ***Trypanosoma brucei rhodesiense***
- ***Trypanosoma cruzi***



Leishmania donovani: amastigotes
red arrow: nucleus; black arrow kinetoplast



Trypanosoma brucei spp
trypomastigote forms

Hemoflagellates: Leishmania

- Protozoan parasites in blood or tissues of humans
  - All species require an arthropod intermediate host as a vector to complete life cycle.
- Leishmania spp
  - Amastigote:
    - Oval, non-flagellated form
    - Primary diagnostic form in humans (with nucleus and kinetoplast)
  - Promastigote:
    - Long, slender form w/ free flagellum
    - Found in arthropod vector, sand fly

General Characteristics: Leishmaniasis

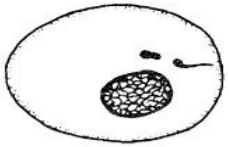
- 20 species capable of causing human disease; most notable mentions are:
  - *L. tropica*
  - *L. mexicana*
  - *L. braziliensis*
  - *L. donovani*
- Leishamaniasis is a zoonotic disease where dogs, rodents, and humans are reservoirs
  - Definitive hosts
- Transmitted by vector – Sand fly
  - Intermediate host to complete lifecycle

General Characteristics: Leishmaniasis

- Signs and Symptoms:
  - Can range from self-healing cutaneous ulcers to visceral disease affecting multiple organs

Morphologic forms of Hemoflagellates

in humans



Amastigote

in vector: Sandfly

Promastigote

Leishmania spp

Amastigote 2-3μm with nucleus and rod-like kinetoplast (dot & dash) in macrophages of liver biopsy splenic puncture, lymph node or bone marrow aspirate

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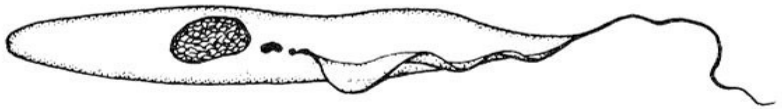
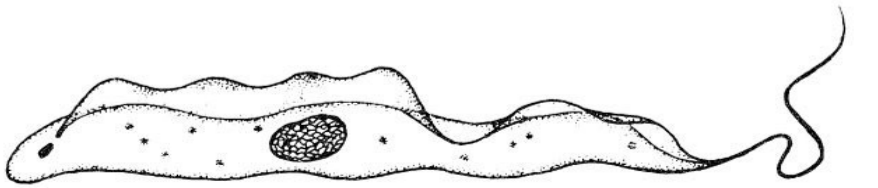
in vector: Tse tse fly or Reduviid bug



Epimastigote

in humans



Trypomastigote

(Illustration by Nobuko Kitamura.)

Trypanosoma spp

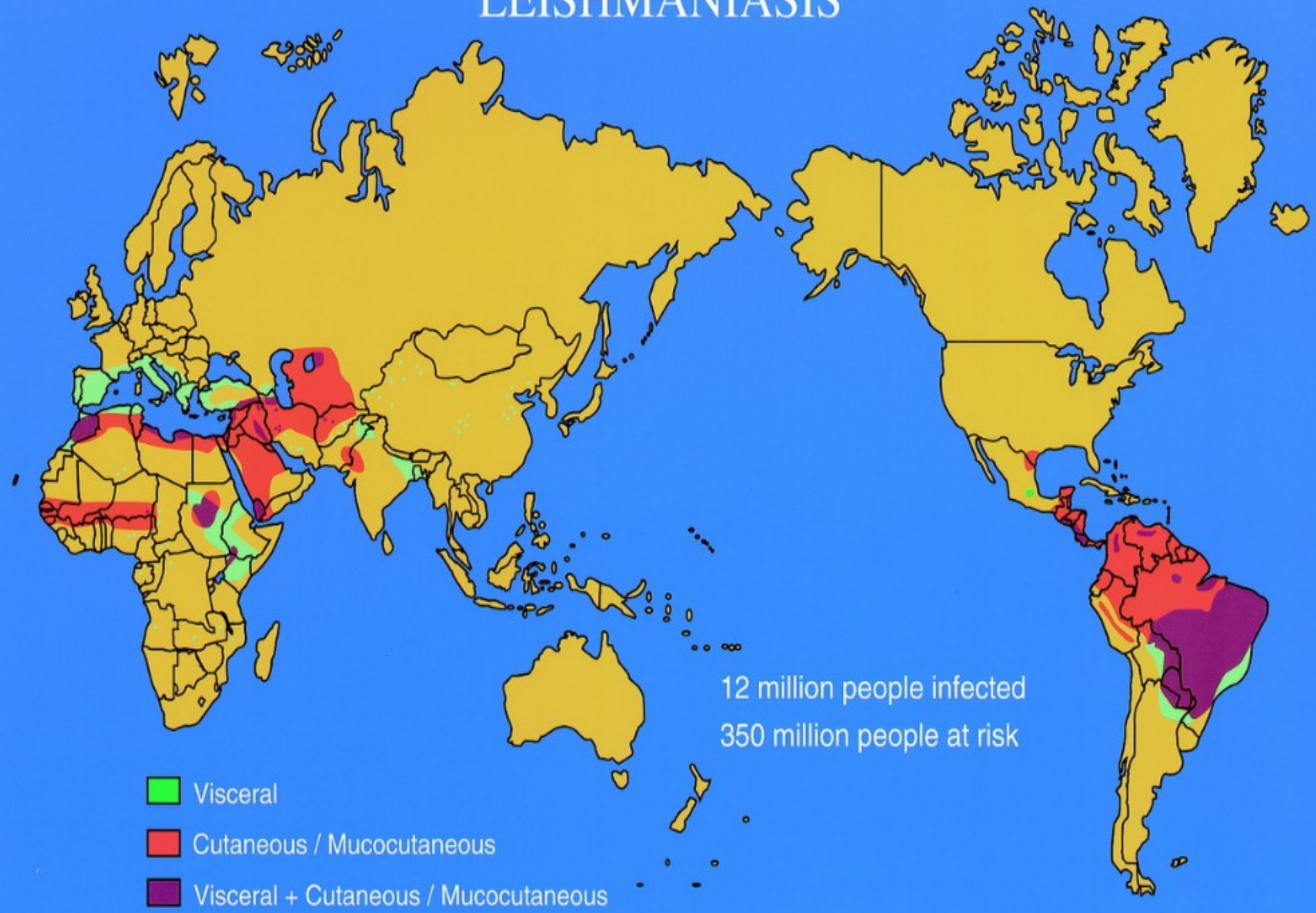
Trypomastigote 14-35μm in blood smear
Amastigote: T. cruzi only

Leishmania spp

Leishmaniasis in Humans

Species	Disease	Geographic Distribution
Leishmania donovani	Visceral leishmaniasis Dermal leishmaniasis (post kala-azar, in India)	Africa (Sudan), Asia (Bangladesh, India, Nepal), and Brazil
L. infantum	Visceral leishmaniasis	Africa, Mediterranean Europe, China, Central & South America
L. tropica	Cutaneous leishmaniasis	Afghanistan, Turkey, India, former USSR, Middle East, Africa
L. major	Cutaneous leishmaniasis	Middle East, Africa, Afghanistan, former USSR
L. mexicana	Cutaneous leishmaniasis Diffuse cutaneous leishm.	Texas, Belize, Guatemala, Mexico
L. braziliensis	Cutaneous leishmaniasis Mucocutaneous leishm.	Central and South America

LEISHMANIASIS



Leishmania donovani

- Most severe version of leishmaniasis
- Most prevalent in South America, Africa, Southern Europe, and Asia
- Causes visceral leishmaniasis is also known as kala-azar or black fever or dumdum fever
- Affects reticuloendothelial system [RES]
  - Liver, spleen, lymph node, and bone marrow

Visceral Leishmaniasis

- Caused by *L. donovani*
- Signs and symptoms:
  - Ranging from asymptomatic, chronic, or fulminant infections
  - Malaria-like spiking chills & fever
  - Sweating
  - Weight loss
  - Hepatosplenomegaly with progressive pancytopenia
  - Death occurs within 2 years if untreated
  - Considered one of most important diseases of humans worldwide

Leishmania donovani

- Lab Values:
  - Elevated liver enzymes
  - Hypogammaglobulinemia
  - Hypoproteinemia
  - Intracellular leishmania in macrophages and histiocytes

Post Kala-azar Dermal Leishmaniasis

- Persistence/chronic visceral leishmaniasis
- Deeply pigmented granulomatous areas of the skin
- Demonstration of amastigotes in macrophages in Wright Geimsa-stained smears
  - Liver biopsy, splenic puncture
  - Aspirates of lymph node or bone marrow
- Nucleic acid amplification tests (NAAT, PCR) for L. donovani DNA from aspirates and biopsy specimens
- Treatment of visceral leishmaniasis
- Pentavalent antimonial compound = Pentostam
- Alternative treatments:
  - amphotericin B liposomal formulation, miltefosine PO, paromomycin IV

Cutaneous Leishmaniasis

- Caused by:
  - L. tropica = “old world or oriental sore” found in far East and North Africa
  - L. mexicana = “new world leishmaniasis” and chiclero ulcers
    - Red crusted circular lesions up to 8 cm in diameter
  - L. braziliensis = found in Central and South America
- Leishmania infiltrate dermal cellular layer, becomes pruritic (itchy)
- Proliferation of amastigotes intracellularly and spread extracellularly
- Recovery with immunity against reinfection
  - Usually self-limiting

Cutaneous Leishmaniasis

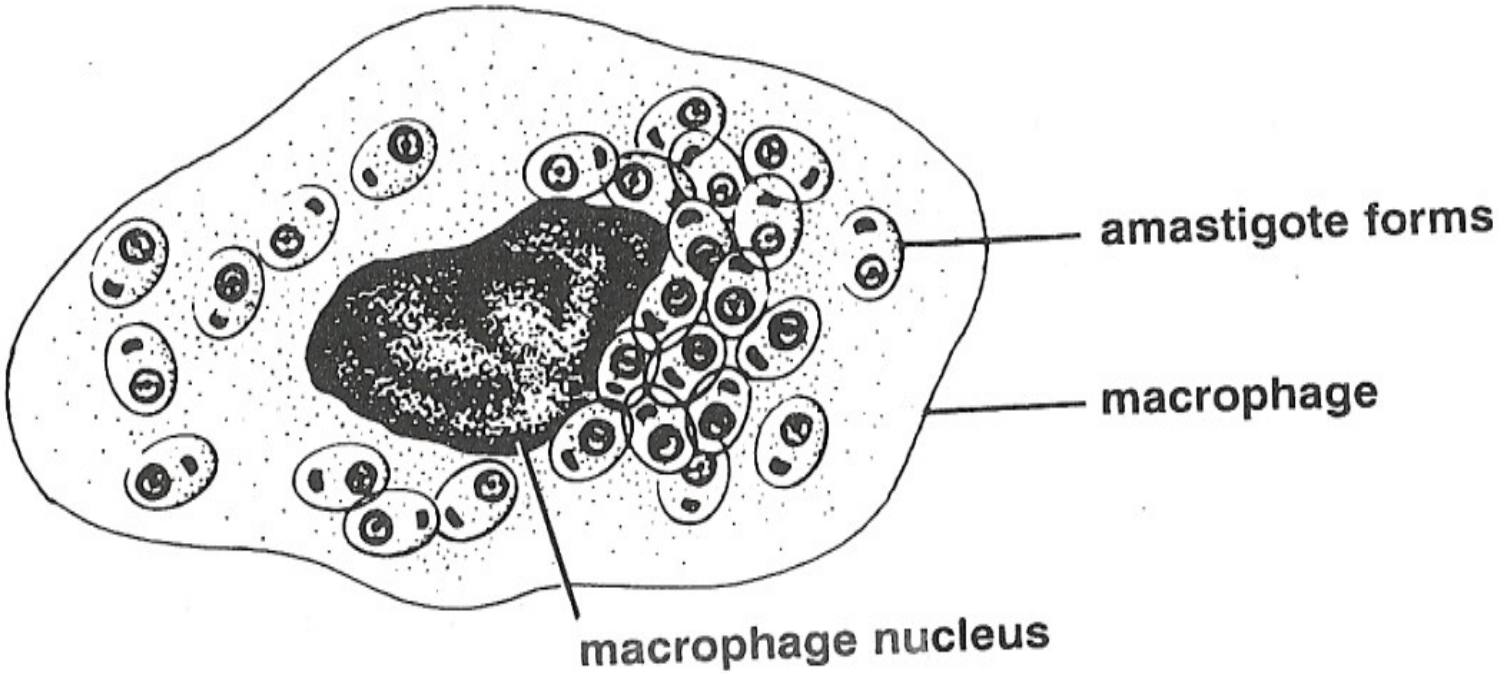
- Diagnosis
  - Amastigotes in stained smears (Giemsa) of touch preps or ulcer biopsy specimens
  - Serology: EIA for IgG & IgM antibody response
  - PCR from biopsy specimens
- Treatments:
  - In simple cutaneous leishmaniasis, lesions heal spontaneously, or topical treatments can be used – cryotherapy, surgical excision
  - For relapses =Pentostam or other pentavalent antimonial compound

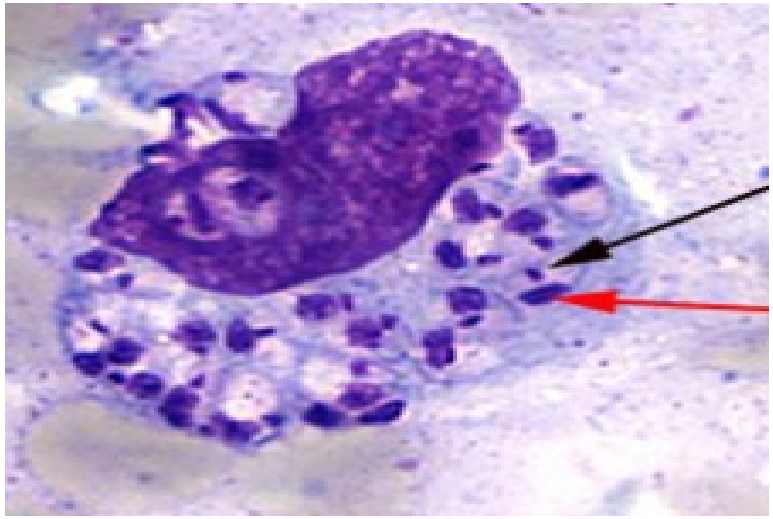
Leishmanial Morphologic Forms

- Amastigote: 3.3 µm long x 2 µm wide
  - Small rounded intracellular morphologic stage in macrophages
  - No flagellum with bar shaped kinetoplast
    - Kinetoplast: = DNA rich structure with basal body near based of flagella in cytoplasm
- Promastigote
  - Morphologic stage in the insect vector
  - Slender flagellum twice the length of the body
  - Kinetoplast located at the anterior end
  - No undulating membrane

DIAGNOSTIC STAGE

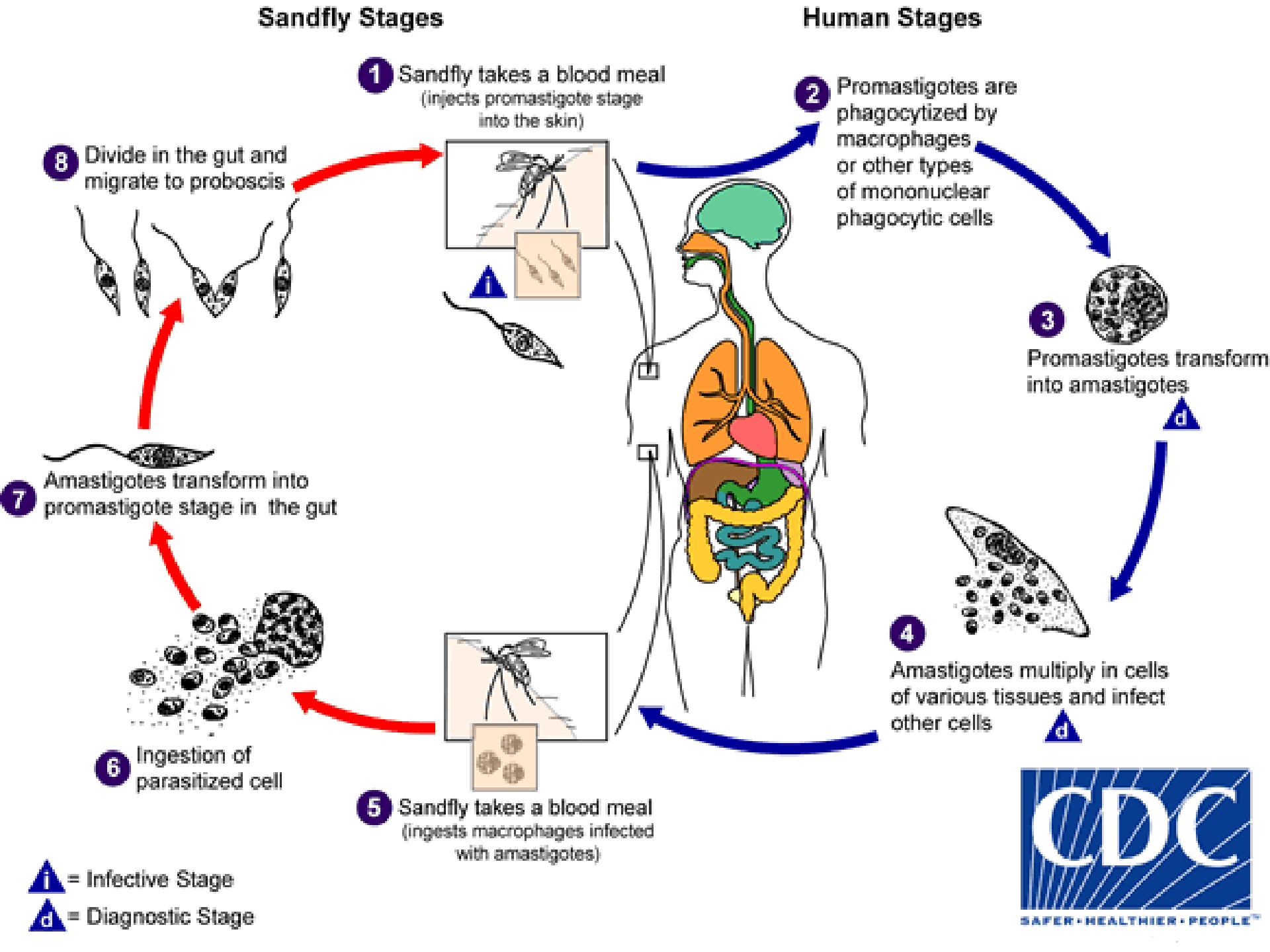
Amastigotes multiplying in tissue macrophages





Leishmanial Generalized Life Cycle

- Female insect takes a blood meal and ingests amastigote stage from reservoir host
  - The amastigote develops into a promastigote in the gut of the insect and migrates to salivary gland
- Promastigote is transmitted to human as insect saliva is injected during the insect's blood meal
  - Promastigote is taken up by receptor mediated phagocytosis in macrophages
- Intracellular promastigote becomes an amastigote and multiplies intracellularly
- Sand fly can take a blood meal and ingest amastigotes to continue the lifecycle.


Sandfly Stages
Human Stages
1 Sandfly takes a blood meal
(injects promastigote stage
into the skin)
2 Promastigotes are
phagocytized by
macrophages
or other types
of mononuclear
phagocytic cells
3 Promastigotes transform
into amastigotes
4 Amastigotes multiply in cells
of various tissues and infect
other cells
5 Sandfly takes a blood meal
(ingests macrophages infected
with amastigotes)
6 Ingestion of
parasitized cell
7 Amastigotes transform into
promastigote stage in the gut
8 Divide in the gut and
migrate to proboscis
i = Infective Stage
d = Diagnostic Stage
CDC
SAFER·HEALTHIER·PEOPLE™

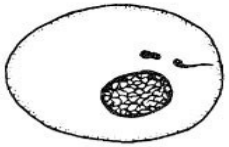
Trypanosoma spp

Hemoflagellates: Trypanosoma

- Protozoan parasites in blood or tissues of humans
  - All species require an arthropod intermediate host as a vector to complete life cycle
- Trypanosoma spp
  - Epimastigote: long, spindle-shaped form with free flagellum and undulating membrane
    - found in arthropod vectors
  - Trypomastigote: long, slender to stubby – C, S, or U shape on permanent stain
    - Has undulating membrane w/ or w/o flagellum
    - primary diagnostic form found in vectors and humans.
- Exception: T. cruzi also has amastigote forms.

Morphologic forms of Hemoflagellates

in humans



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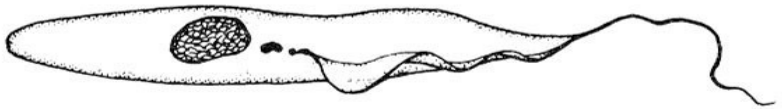
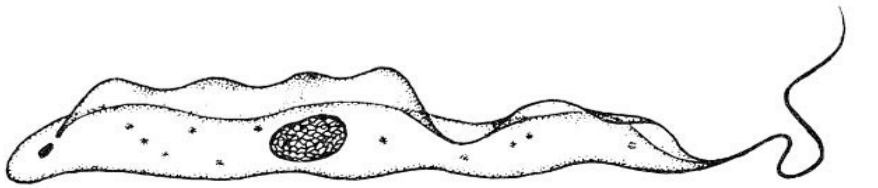


Epimastigote

in humans



Trypomastigote

(Illustration by Nobuko Kitamura.)

Trypanosoma spp

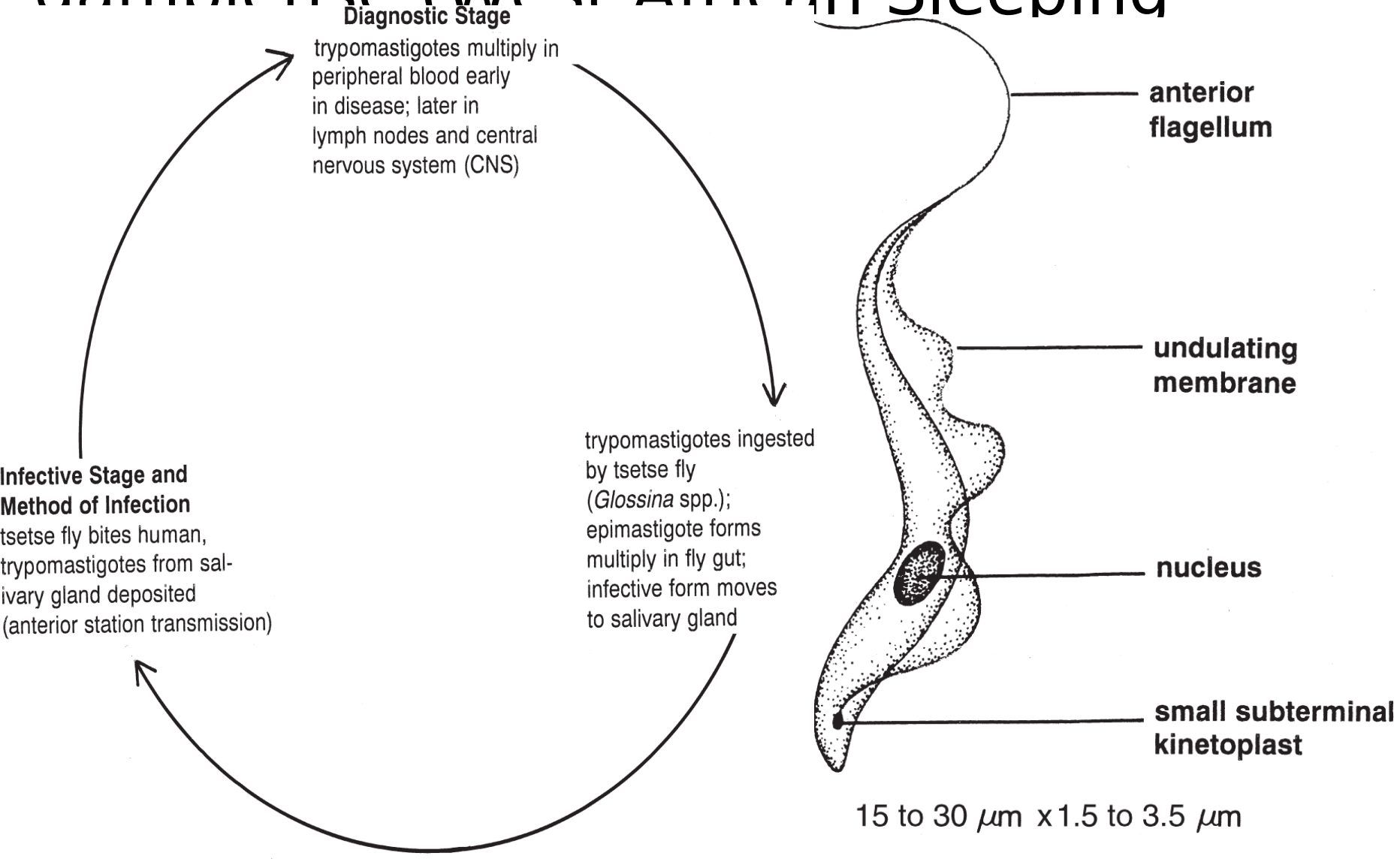
Trypomastigote 14-35μm in blood smear
Amastigote: T. cruzi only

Trypanosomiasis

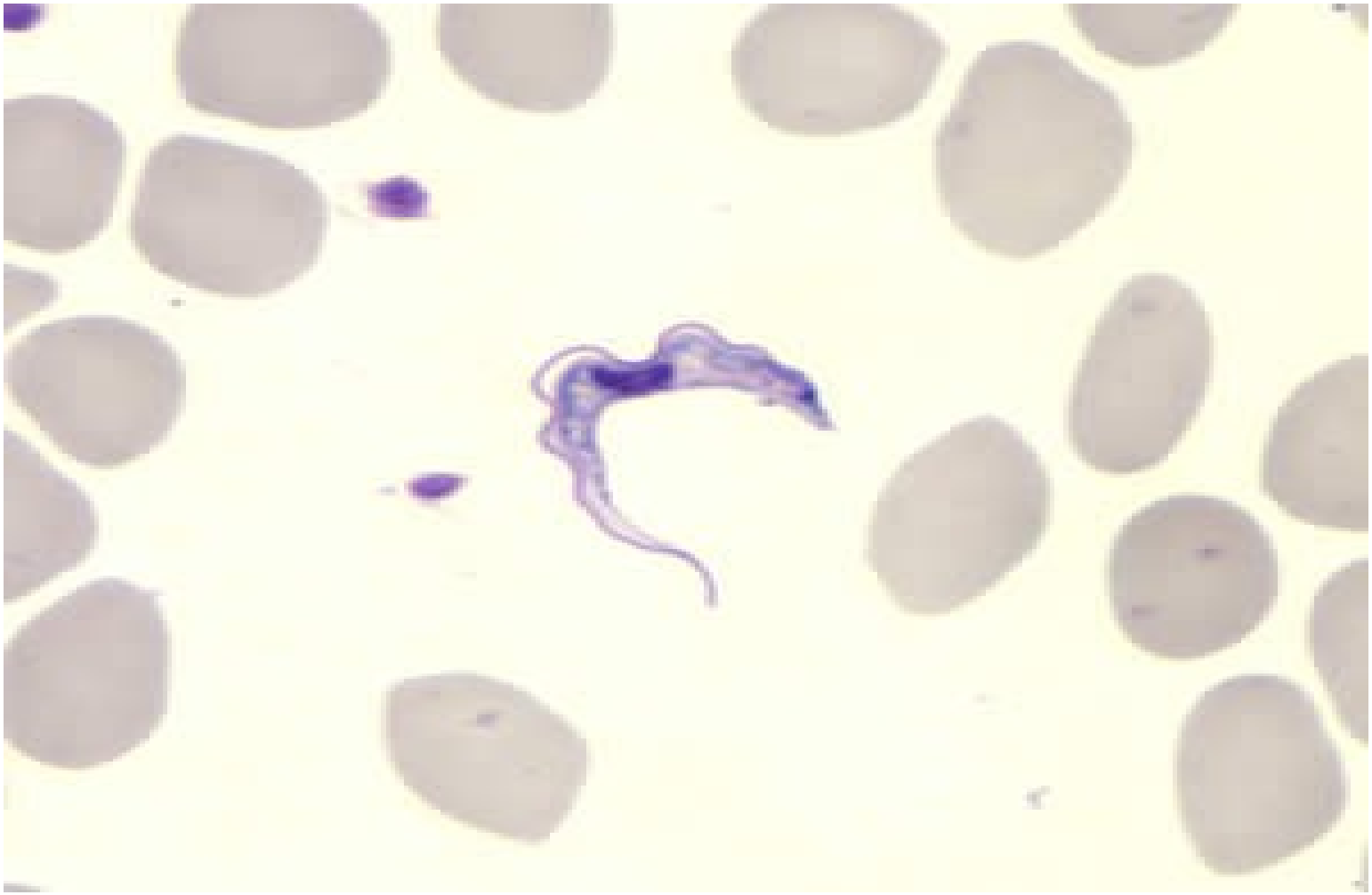
- Three Important species of Trypanosomes:
- African Sleeping Sickness:
  1. *Trypanosoma brucei rhodesiensis*
     - East African sleeping sickness; vector – tsetse fly
     - Parasites of wild herbivores but infect humans given the opportunity
  2. *Trypanosoma brucei gambiense*
     - West African sleeping sickness; vector – tsetse fly
- Chagas Disease [American Trypanosomiasis]
  3. *Trypanosoma cruzi*
     - Zoonotic disease that can be transmitted to human by blood-sucking reduviid bugs (triatome bugs)
     - Found in South and Central America; isolated incidence in US

T. brucei

brucei
rodesiense (East African Sleeping Sickness)
gambiense (West African Sleeping



Trypanosoma brucei spp



Trypanosomiasis

- Virulence of Trypanosomes come from trypomastigote outer membrane polymorphism with ability to change the outer membrane coat.
  - Changes every 5 -7 days and the host immune system must start over
- African sleeping sickness
  - Local inflammatory reaction at the site of the insect bite
    - Lasts up to 4 weeks
  - Stage 1: hemo-lymphatic phase
    - 1-3 weeks after initial infection
    - Trypomastigotes enter the blood and lymphatic fluid
  - Stage 2: meningoencephalitis:
    - Trypomastigotes invade CNS
    - Neurological problems, coma, death

Trypanosomiasis: **West** African Sleeping Sickness

- *Trypanosoma brucei gambiense*
- Endemic in tropical rainforest of Central and Western Africa
  - Humans are primary reservoir
- Signs and Symptoms:
  - Lymphadenopathy in posterior cervical region of the neck = Winterbottom's sign
  - headaches, night sweats, joints & muscle pains
- Invasion of CNS produces the sleeping sickness
  - behavioral and personality changes
  - fatigue, apathy, confusion, somnolence
  - signs of progressive meningoencephalitis; coma and death
- Invariably fatal within 2 to 7 years if left untreated
  - CHRONIC

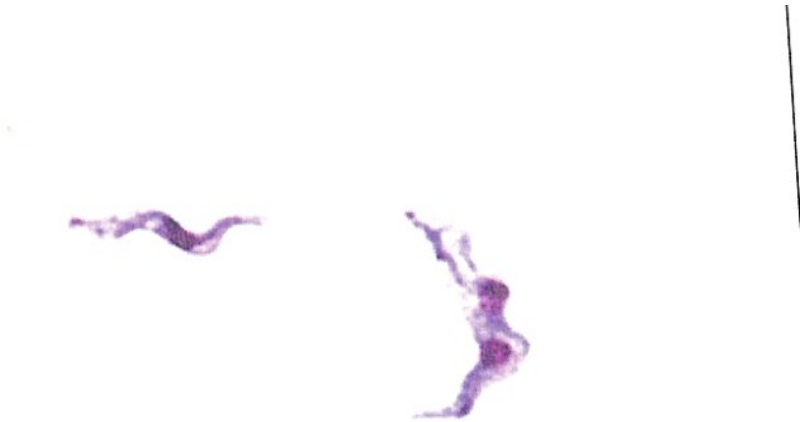
Trypanosomiasis: **East** African Sleeping Sickness

- *Trypanosoma brucei rhodesiense*
- Rapid onset and death within 6 months
  - ACUTE
- Zoonotic and human reservoirs
- Endemic in the savannah and woodland regions of Central and East Africa
- Same lifecycle and disease progression just faster


Tunisia
Morocco
Western Sahara
Algeria
Libya
Egypt
Mauritania
Mali
Niger
Chad
Sudan
Eritrea
Djibouti
Cape Verde
Gambia
Sierra Leone
Guinea Bussau
Liberia
Côte d'Ivoire
Togo
Ethiopia
Somalia
Kenya
Equatorial Guinea
Sao Tome and Principe
Angola
Malawi
Zimbabwe
Madagascar
Mozambique
Namibia
Botswana
Swaziland
Lesotho
South Africa
West African trypanosomiasis
East African trypanosomiasis

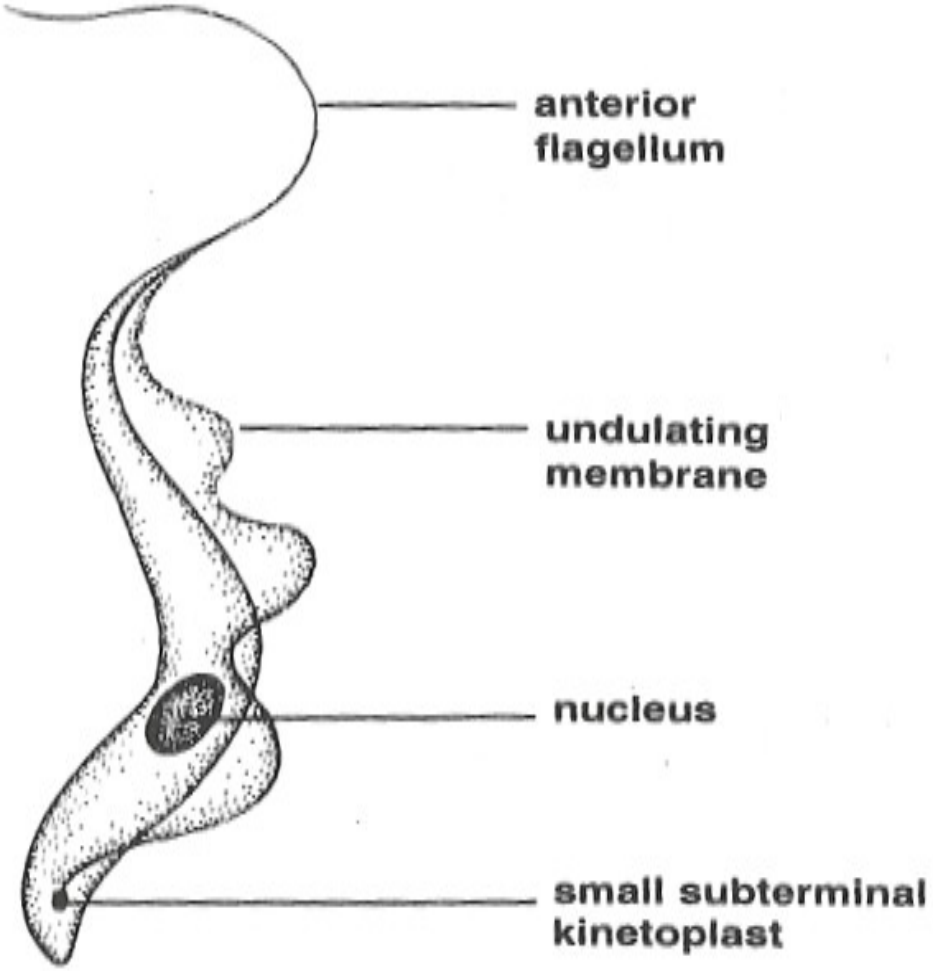
African Trypanosomiasis

- Trypomastigotes
- T. b. gambiense & T. b. rhodesiense are indistinguishable
- Dividing form is larger than the nondividing one
- Trypomastigotes are 15-20 um long with a single large nucleus and a posterior kinetoplast
  - Flagellum with undulated membrane is attached to kinetoplast



DIAGNOSTIC STAGE

Trypomastigote form in plasma



15 to 30 μm x 15 to 3.5 μm

Note: Trypomastigote form may be seen dividing in peripheral blood.

African Trypanosomiasis: Life Cycle

- The entire life cycle of African trypanosomes is represented by extracellular stages.
- The cycle in the fly takes approximately 3 weeks
- Humans are the main reservoir for T. b. gambiense
- Wild game animals are reservoir for T. b. rhodesiense

Tsetse fly (Glossina spp)

African Trypanosomiasis: General Life Cycle

- Tsetse fly is a biological vector
- Fly ingests trypomastigote stage during human blood meal
- Trypomastigote migrate to Tsetse fly intestinal area and become Epimastigote
  - Epimastigote migrate to salivary glands of fly
  - Develop into metacyclic trypomastigotes
- Metacyclic trypomastigotes are transmitted to humans during blood meal

Tsetse fly (Glossina spp)

African Trypanosomiasis

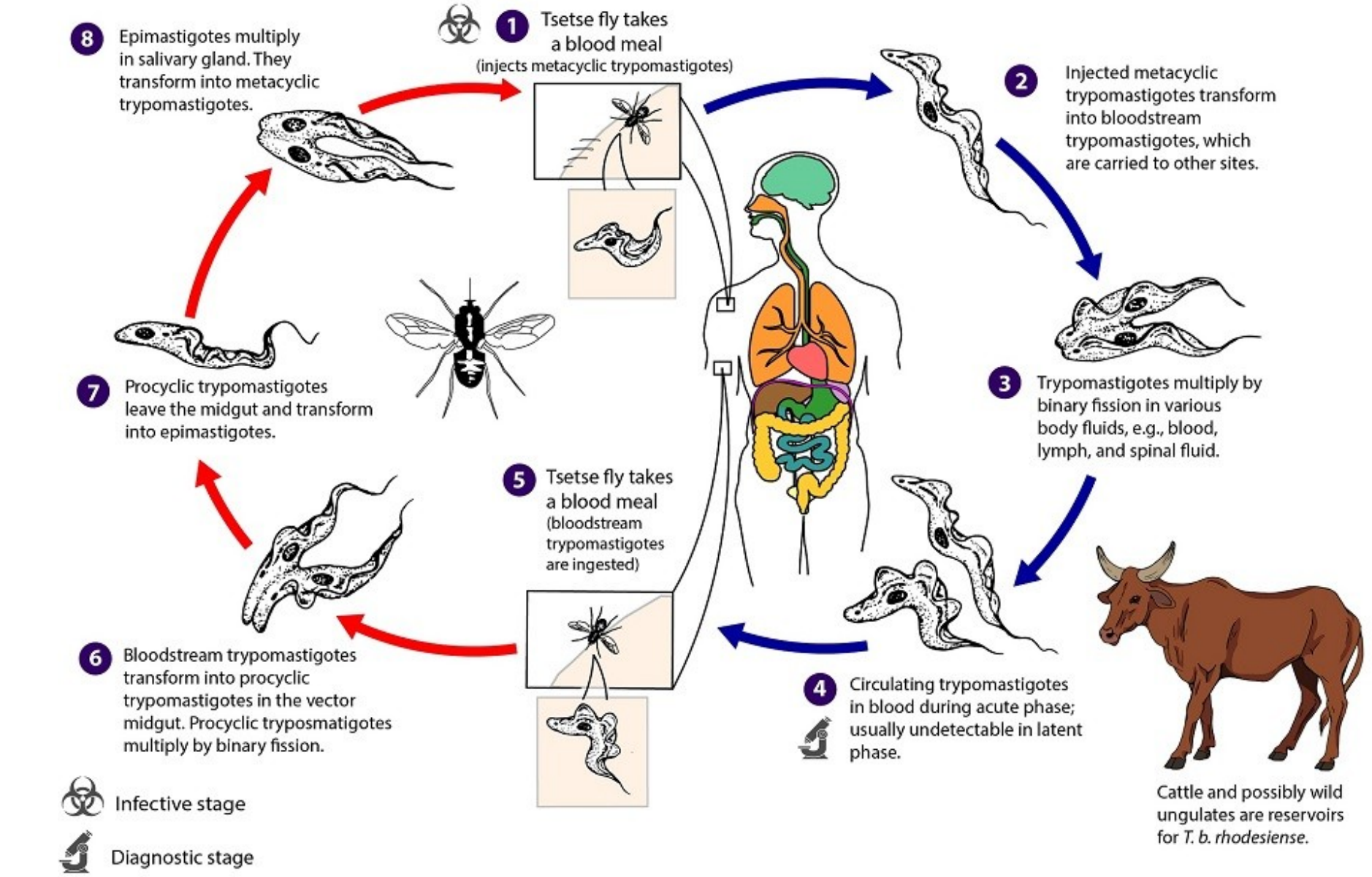
Trypanosoma brucei gambiense & Trypanosoma brucei rhodesiense

DPDx

CDC

Tsetse Fly Stages

Mammalian Stages



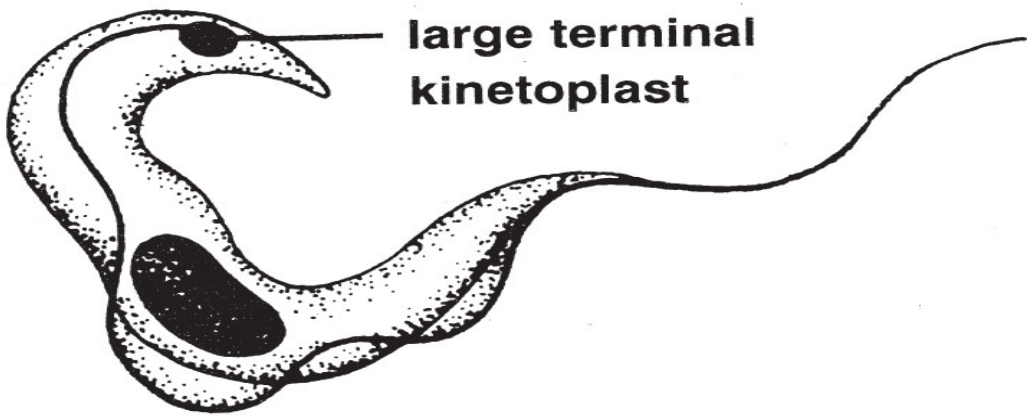
African Trypanosomiasis: Lab Diagnosis

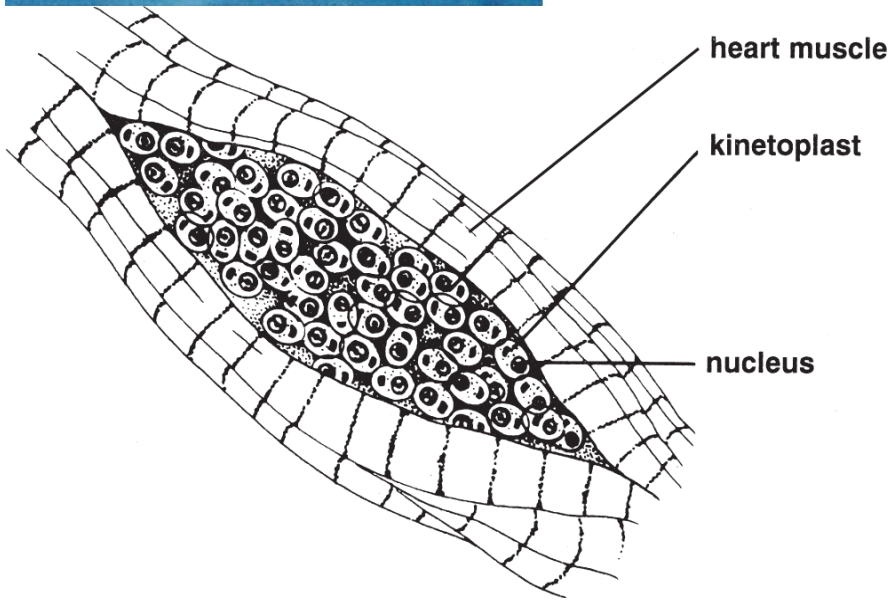
- Wright-stained blood smear or thick and thin blood smear
- May also be detected in lymphatic fluid and CSF
- Trypomastigotes found in blood (Speciation cannot be determined)
- CBC will have increased lymphocytes
- BMP/CMP will show Elevated serum and fluid protein
- QBC method – Quantitative Buffy Coat with Acridine Orange
- Serological Card Agglutination Test [CATT]
  - Lyophilized T. gambiense antigen detects preformed antigen from human serum
  - Cannot determine active vs chronic vs past infections
- ELISA
- Nothing for T brucei rhodesiense.

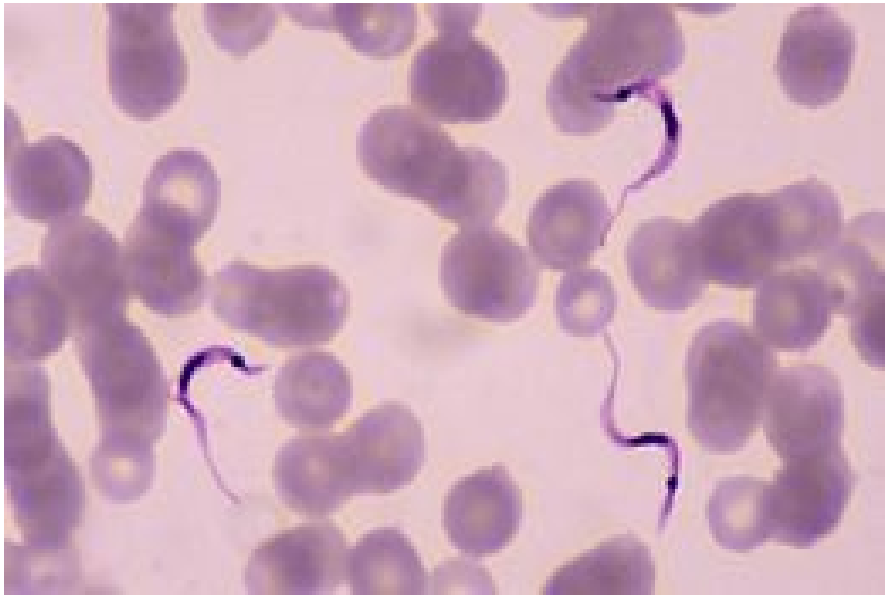
T. cruzi

Diagnostic Stage of T. cruzi

B C- or S-shaped trypomastigote form in blood







American Trypanosomiasis: Chagas Disease

- Zoonotic infection commonly seen in Central and South America
- Isolated incidents in the US have occurred, but could get worse with climate change
- Caused by T. cruzi
- Transmission through feces of reduviid bug getting into its bite wound
  - Also known as kissing bug or triatomid bug
  - Armadillos and opossums may also be reservoirs
- Reduviid bugs live in mud and come out at night to feed
- It is possible to be transmitted by blood products which are why Chagas is tested for during blood donation processes

American Trypanosomiasis: Chagas Disease

- Clinical disease has acute and latent/chronic phases
- Initial bite has incubation period of 2-4 weeks
- Acute phase may have chagoma
  - Ulcerative skin lesion at the site of insect bite
  - Romana sign – unilateral edema around the eye
  - Asymptomatic to fever, lymphadenitis, hepatosplenomegaly, N/V/D
  - Myocarditis and meningitis can also occur

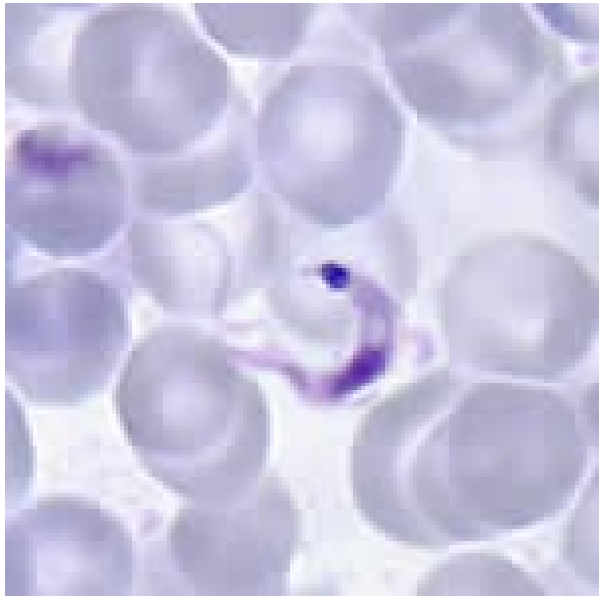
American Trypanosomiasis: Chagas Disease

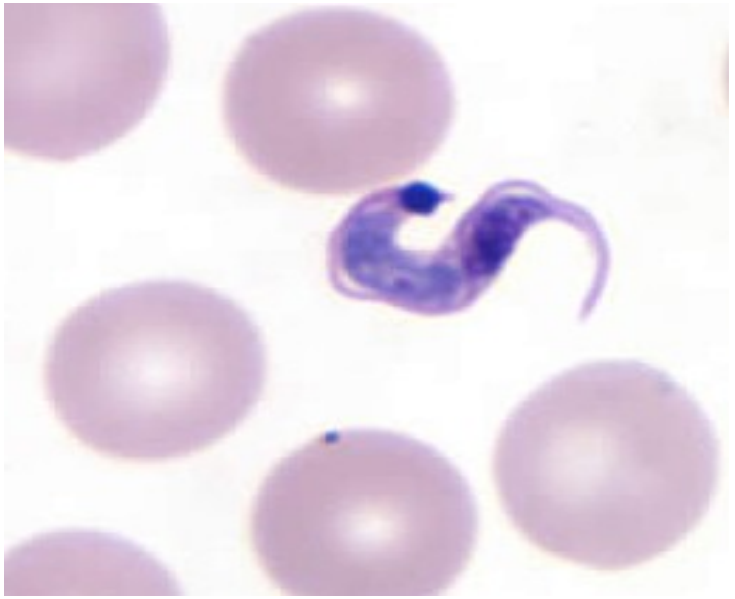
- Clinical disease has acute and latent/chronic phases
- Latent/chronic
  - Trypomastigotes disappear from circulation and invade heart and GI cells
  - Can last 10 – 40 years but only an average of 35% of infected people develop chronic Chagas disease
    - Tachycardia, CHF, Myocarditis
    - Megacolon
      - Affects peristalsis and digestion
  - Damage to organs can be from the parasite or the host's immune response
    - Interferons, cytokines, autoantibodies, inflammation

Trypanosoma cruzi: Chagas Disease

American Trypanosomiasis: Morphology

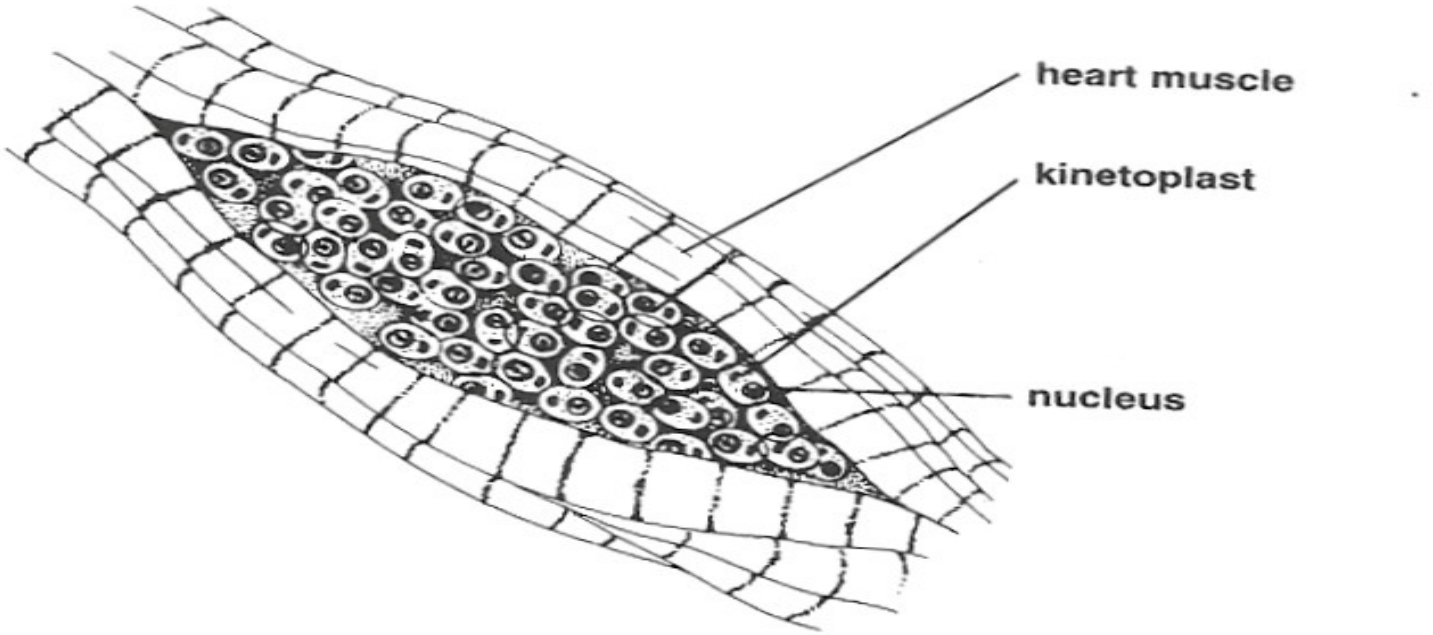
- Slender forms of T. cruzi- usually associated w/ acute infection
- **C-shaped trypomastigotes** (4 C's: cruzi, Chagas, cardiac, C-shaped)
- Epimastigote in stained preparations from cultures



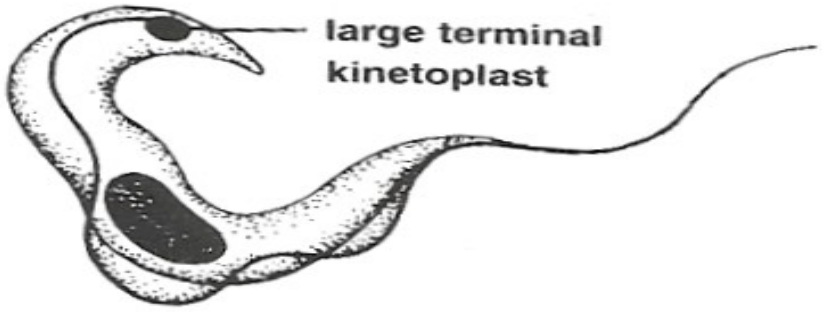


DIAGNOSTIC STAGE

Pseudocyst containing amastigote stages in heart muscle



C- or S-shaped trypomastigote form in blood



Note: Trypomastigote forms do not divide in blood.

American Trypanosomiasis: Life Cycle

- Reduviid bugs feed at night (nocturnal)
- T. cruzi can cross the placenta and can cause disease prenatally
- Can be transmitted through blood transfusions, organ transplantation and in laboratory accidents

American Trypanosomiasis: Life Cycle

- Transmission from Reduviid bug to humans occurs after insect vector bites and defecates in the area surrounding the bite wound
- Metacyclic trypomastigotes in its feces are scratched into the bite wound
- Trypomastigotes enter the blood stream and enter cells
  - Heart and GI
  - Develops into amastigotes
- Amastigotes multiply and the cell ruptures and amastigotes invade other cells
- Some amastigotes become trypomastigotes which can be taken back into insect vector following a blood meal to continue the cycle

Trypanosoma cruzi

DPDx

Triatomine Bug Stages

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

8. Metacyclic trypomastigotes in hindgut

7. Multiply in midgut

6. Epimastigotes in midgut

5. Triatomine bug takes a blood meal
(trypomastigotes ingested)

Mammalian Stages

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.

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

Trypomastigotes can infect other cells and transform into intracellular amastigotes in new infection sites.

Many mammalian species have been recognized as *T. cruzi* reservoir hosts.

CDC

Infective stage

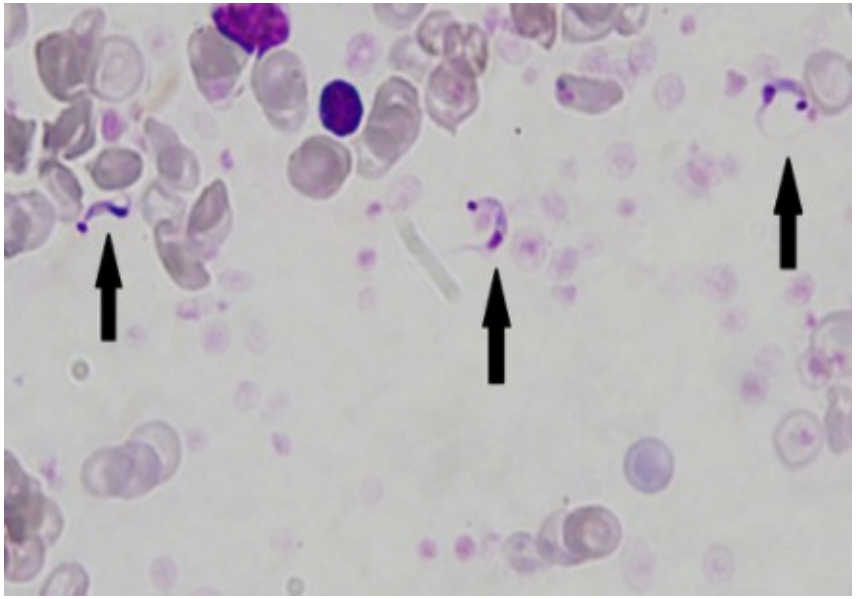
Diagnostic stage

American Trypanosomiasis: Chagas Disease
Lab Diagnosis and ID

- Trypomastigote identified in the blood
- Elongated hemoflagellate that is 15 – 20 um in size
- Often C or S or U shaped
- Single large nucleus midbody
- Anterior flagellum with posterior kinetoplast attached to undulating membrane
- Tissue biopsy may reveal amastigote stage
- Complete patient history is important to determine speciation
- In endemic areas, xenodiagnoses of lab raised reduviid bugs can feed on patients suspected of *T. cruzi*
  - The insect poop is analyzed on a regular basis for the present of metacyclic trypomastigotes to confirm the patient has the disease

Review Question 1

- A 46 year-old male was admitted to a hospital in Southern California presenting with fever and shortness of breath. Radiographs revealed an enlarged heart and a complete blood count smear results showed rare C-shaped trypomastigote forms. The patient had recently immigrated from Brazil. The most likely parasite observed on the Wright-Giemsa stain was:
- a. *Babesia microti*
- b. *Leishmania donovani*
- c. *Trypanosoma brucei gambiense*
- d. *Trypanosoma cruzi*



Homework

- For each of the following parasites, name the morphologic form found in humans, state where this form is found in the infected host, and describe the pathology that the organism produces.
- *L. tropica:*
- *L. braziliensis:*
- *L. donovani:*
- *T. cruzi:*
- *T. brucei:*
- Name the insect vector in the *Leishmania* spp. life cycle and describe the morphologic form found in the vector.

- Name the insect vector for T. brucei

- Name the insect vector for T. cruzi