

Parasitology LABORATORY

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- ▶ Reliability
 - ▶ Proper specimen collection
 - ▶ Proper handling/processing
 - ▶ Accurate reading and reporting
 - ▶ Skilled reader
 - ▶ Quality of equipment

Portals of Entry

- ▶ Mouth
 - ▶ Ingestion of infective stage
 - ▶ Embryonated eggs
 - ▶ Protozoan cysts
 - ▶ Metacercariae
- ▶ Skin
 - ▶ Active larval penetration
 - ▶ Filariform larva
 - ▶ Cercariae
 - ▶ Arthropod introduction

- ▶ Others
 - ▶ Sexual intercourse
 - ▶ Transplacental
 - ▶ Transmammary
 - ▶ Intranasal

Portal/s of Exit

- ▶ Anus
 - ▶ Stool
 - ▶ Eggs
 - ▶ Larvae
 - ▶ Adults, scolex, segments
 - ▶ Cysts
 - ▶ Trophozoites

Specimens for Diagnosis

- ▶ Stool
- ▶ Urine
 - ▶ *Schistosoma haematobium*
 - ▶ *Trichomonas vaginalis*
 - ▶ Filarial worm
- ▶ Sputum
 - ▶ *Paragonimus* egg
 - ▶ *Strongyloides*
- ▶ Blood
- ▶ Tissue biopsies
 - ▶ *Trichinella spiralis*
 - ▶ *Taenia solium*
- ▶ Orifice swab
 - ▶ Vaginal swab
 - ▶ Peri-anal swab

THE FLAGELLATES

Phylum Sarcomastigophora

Subphylum Mastigophora

- ▶ *Chilomastix mesnili*
- ▶ *Retortamonas intestinalis*
- ▶ *Giardia lamblia*
- ▶ *Trichomonas vaginalis*
- ▶ *Trypanosoma spp.*
- ▶ *Leishmania spp.*

- ▶ Morphology
- ▶ Life Cycle
- ▶ Pathology, Symptoms
- ▶ Diagnosis
- ▶ Treatment

Chilomastix mesnili

- ▶ World-wide distribution
- ▶ Found in cecum and colon of man, pigs, chimpanzees and monkeys
- ▶ Water-borne
- ▶ Harmless commensal

Chilomastix mesnili

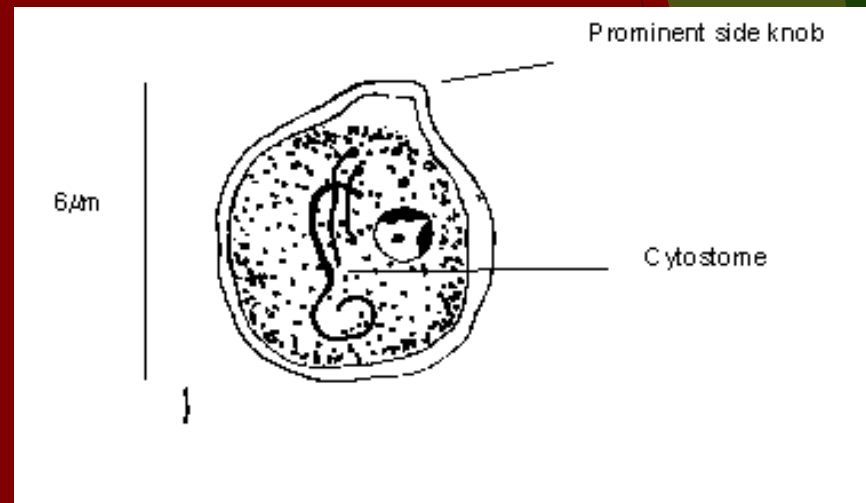
▣ Trophozoite

- 6-24 μ m long and 3-20 μ m wide
- asymmetrical pear-shaped
- nucleus w/ central karyosome and achromatic fibrils
- cytostome
- 3+1 flagella
- Granular cytoplasm



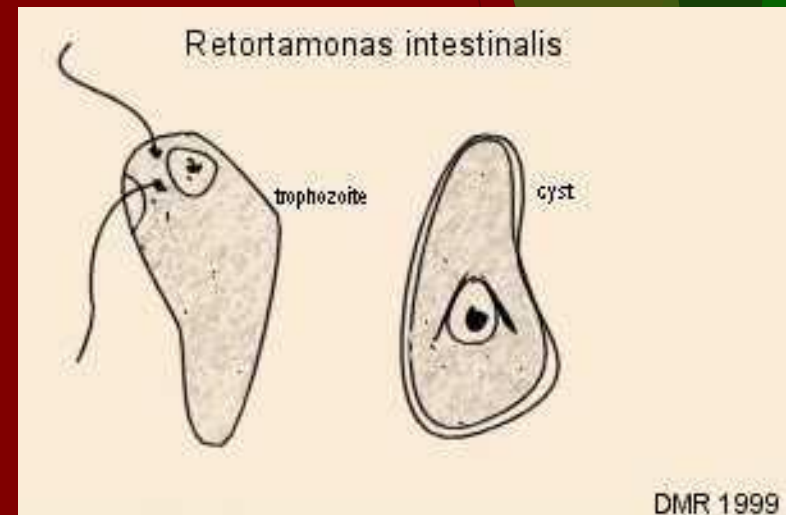
▣ Cyst

- Lemon shape with anterior protrusion
- uninucleated



Retortamonas intestinalis

- ▶ Trophozoite
 - ▶ 4-9 μm
 - ▶ Biflagellated
- ▶ Cyst
 - ▶ 4 μm
 - ▶ Uninucleated
- ▶ Commensal in human intestine
- ▶ Infection occurs through ingestion of cysts in fecal-contaminated food or water, or on fomites



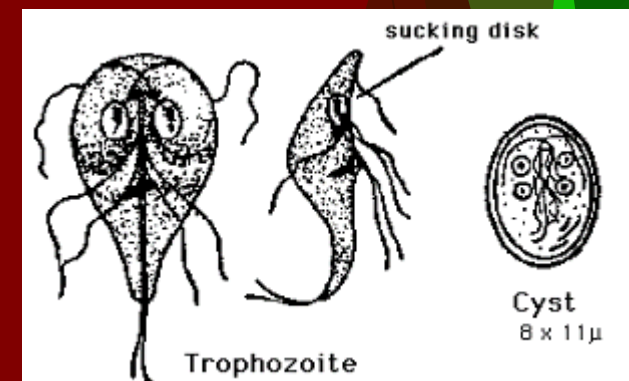
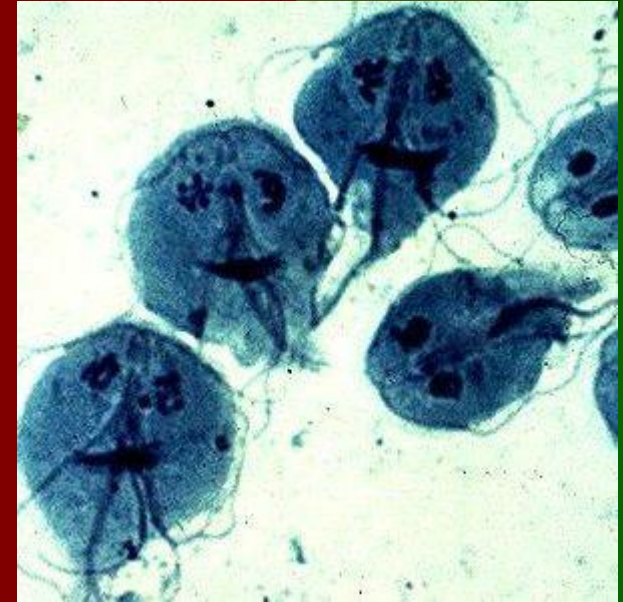
Giardia lamblia

- Discovered in 1681 by Leeuwenhoek
- Described by Lambl in 1859 - *Cercomonas intestinalis*
- Renamed by Stiles in 1915
- Giardiasis
- Worldwide distribution
- Giardiasis
 - Gastrointestinal disease
 - Not life threatening
 - Poor environmental condition
 - Prevalence
 - Higher among children, and practicing oro-anal sex

Giardia lamblia

▶ Trophozoite

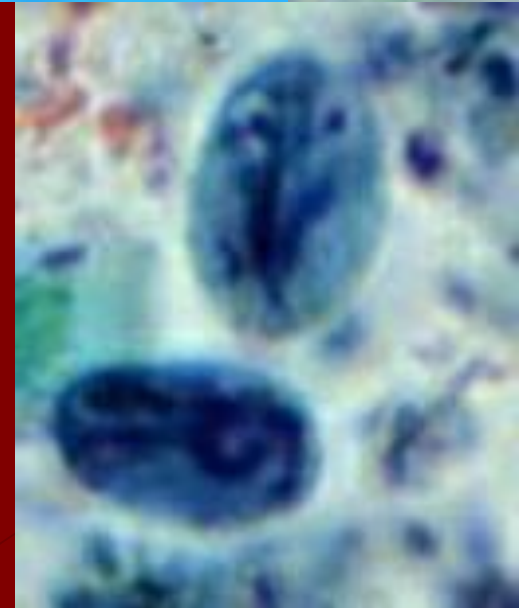
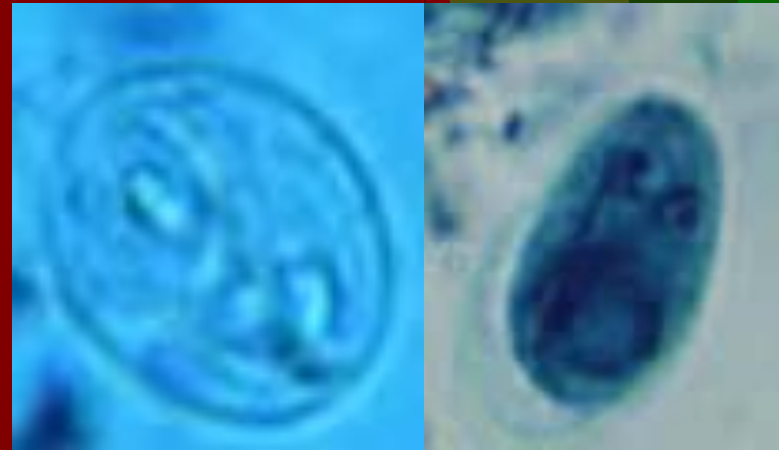
- ▶ 12-15µm long; 5-15 µm wide
- ▶ Pyriform-shaped
- ▶ 2 ovoid nuclei
- ▶ Ventral side concave with adhesive disc
- ▶ Axostyle
- ▶ 4 pairs of flagella
- ▶ Longitudinal binary fission
- ▶ Diarrheic stool
- ▶ Variant surface specific proteins (VSPs) - resistant to intestinal proteases



Giardia lamblia

▶ Cyst

- ▶ Ovoid
- ▶ 8-12 μm long, 7-10 μm wide
- ▶ 2- 4 nuclei
- ▶ Flagella retracted
- ▶ Hyaline cyst wall
- ▶ Infective stage
- ▶ Reproductive encystation
- ▶ Formed stool

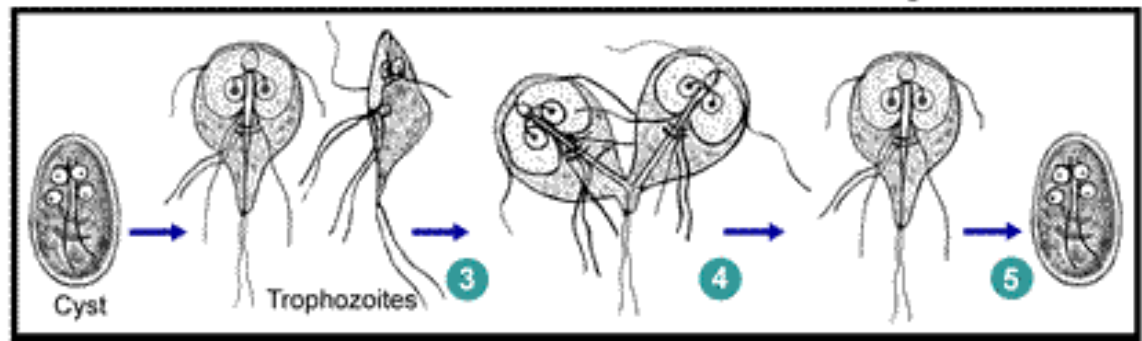
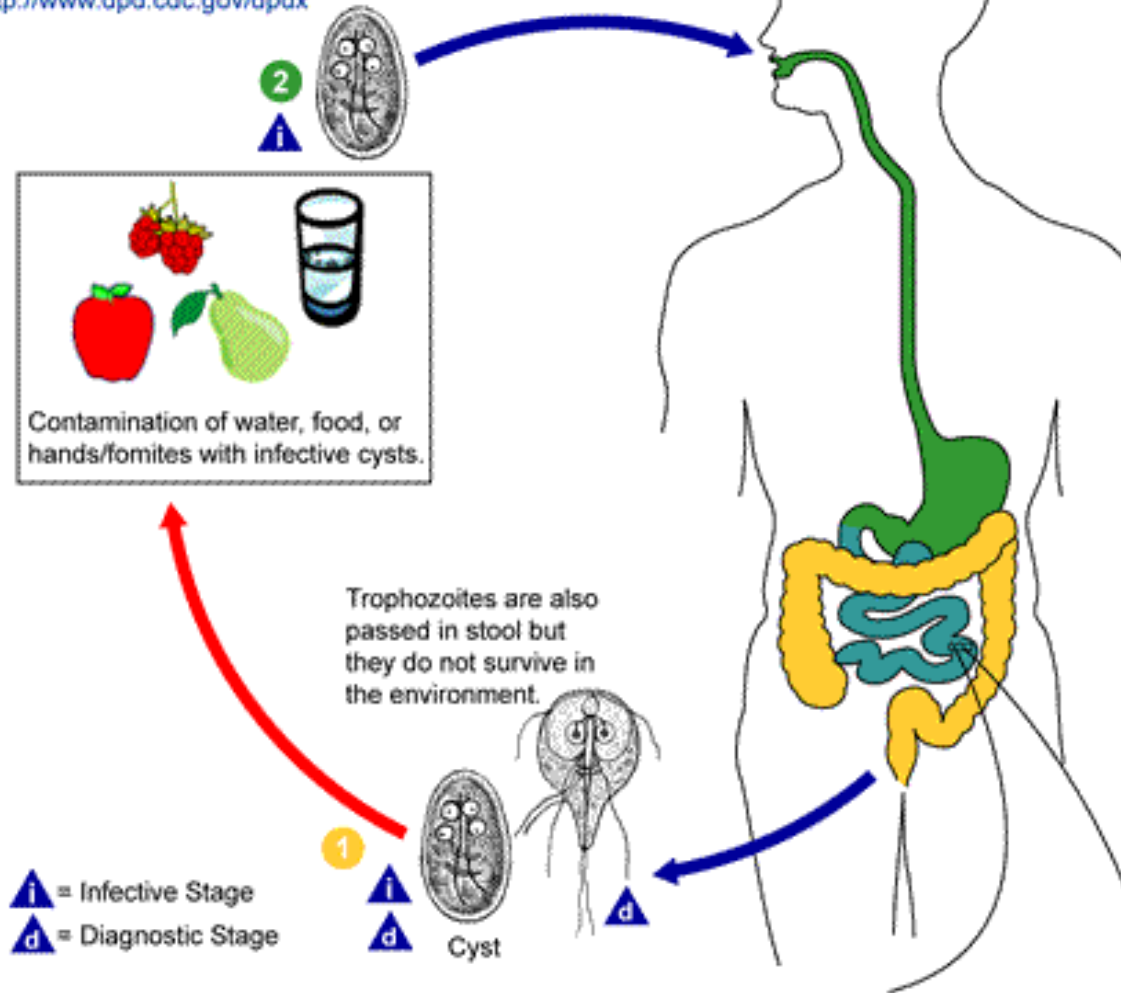


Giardia lamblia



SAFER • HEALTHIER • PEOPLE™

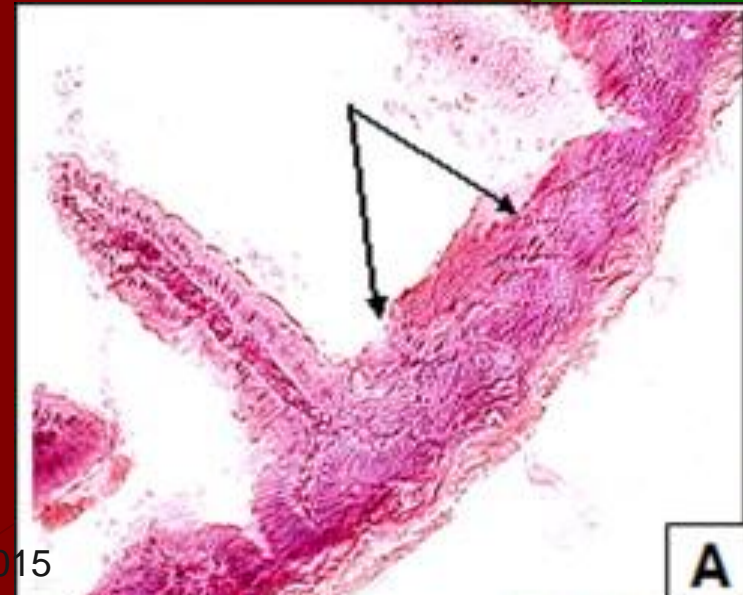
<http://www.dpd.cdc.gov/dpdx>



Giardia lamblia

▶ Pathology

- ▶ Infection dose - as few as 10 cysts
- ▶ Infected person might shed 1-10 billion cysts daily in their feces which could last for several months
- ▶ Adhesive disc attachment (pH 7.8-8.2, lectin) to enterocyte; → avoid peristalsis, irritation of tissues
- ▶ Flattened mucosa - decreased SA for absorption



Giardia lamblia

▶ Symptoms

- ▶ 50% asymptomatic
- ▶ Nausea, anorexia
- ▶ Low-grade fever
- ▶ Explosive, foul-smelling diarrhea

▶ Diagnosis

- ▶ Direct fecal smear (DFS)
- ▶ ELISA, PCR, IFA

▶ Treatment

- ▶ Metronidazole

Molecular Characterization

- Subdivisions of *G. lamblia* based on molecular analysis into different genetic assemblages (A,B,C,D,E,F, and G), with subtypes: A-I, A-II, A-III, A-IV.

Assemblages	Some Species Commonly Infected
A-I	Humans and animals (cats, dogs, livestock, deer, muskrats, beavers, voles, guinea pigs, ferrets)
A-II	Humans (more common than A-I)
A-III and A-IV	Exclusively animals
B	Humans and animals (livestock, chinchillas, beavers, marmosets, rodents)
C and D	Dogs, coyotes
E	Cattle, goats, pigs, sheep
F	Cats

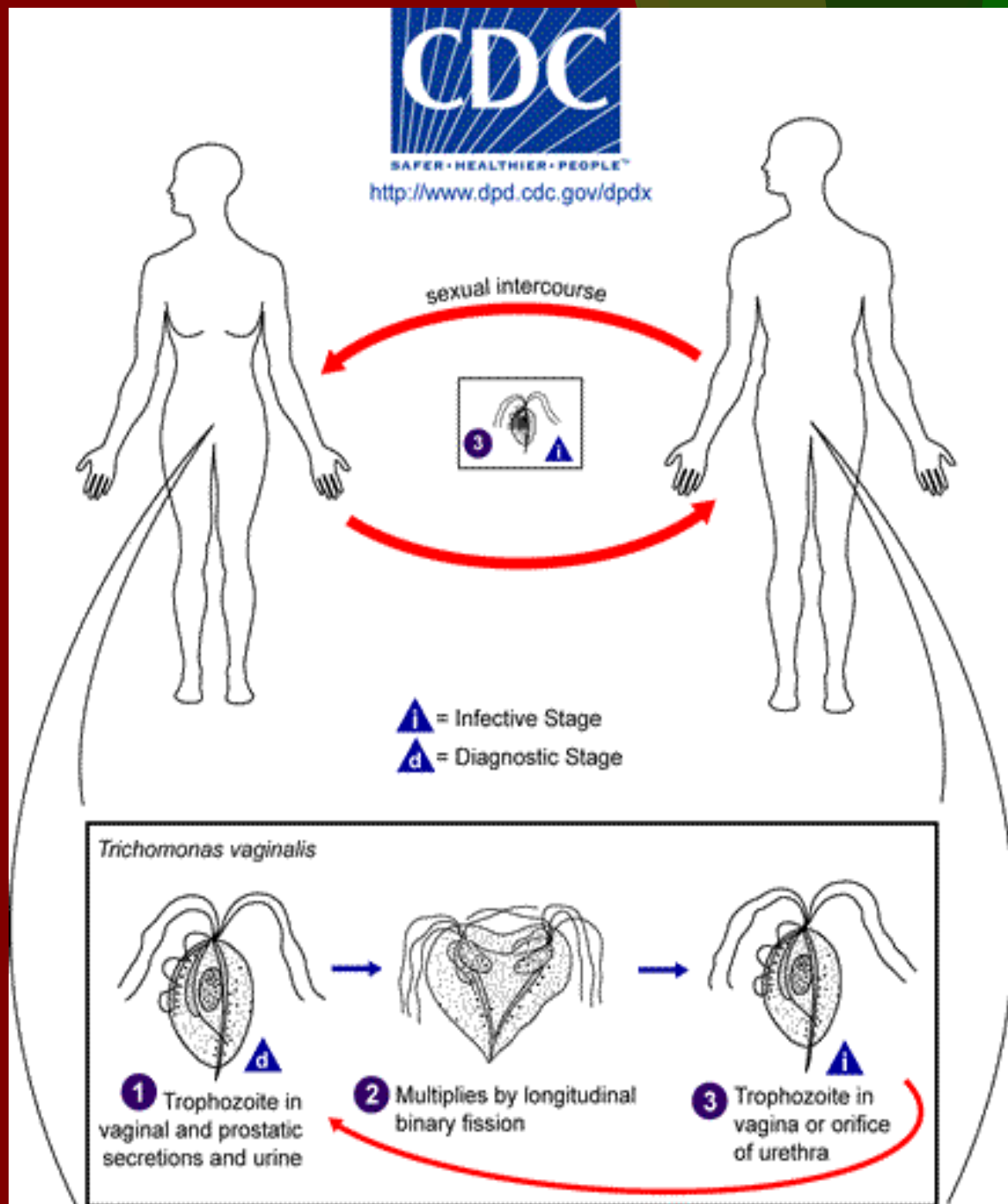
Source: CDC

Trichomonas vaginalis

- ▶ Trichomoniasis
- ▶ Trophozoite
 - ▶ Wet mount: 5-18 μm
 - ▶ 4 free flagella + 1 along undulating membrane
 - ▶ Large nucleus with evenly distributed chromatin
 - ▶ Hydrogenosomes
 - ▶ Axostyle

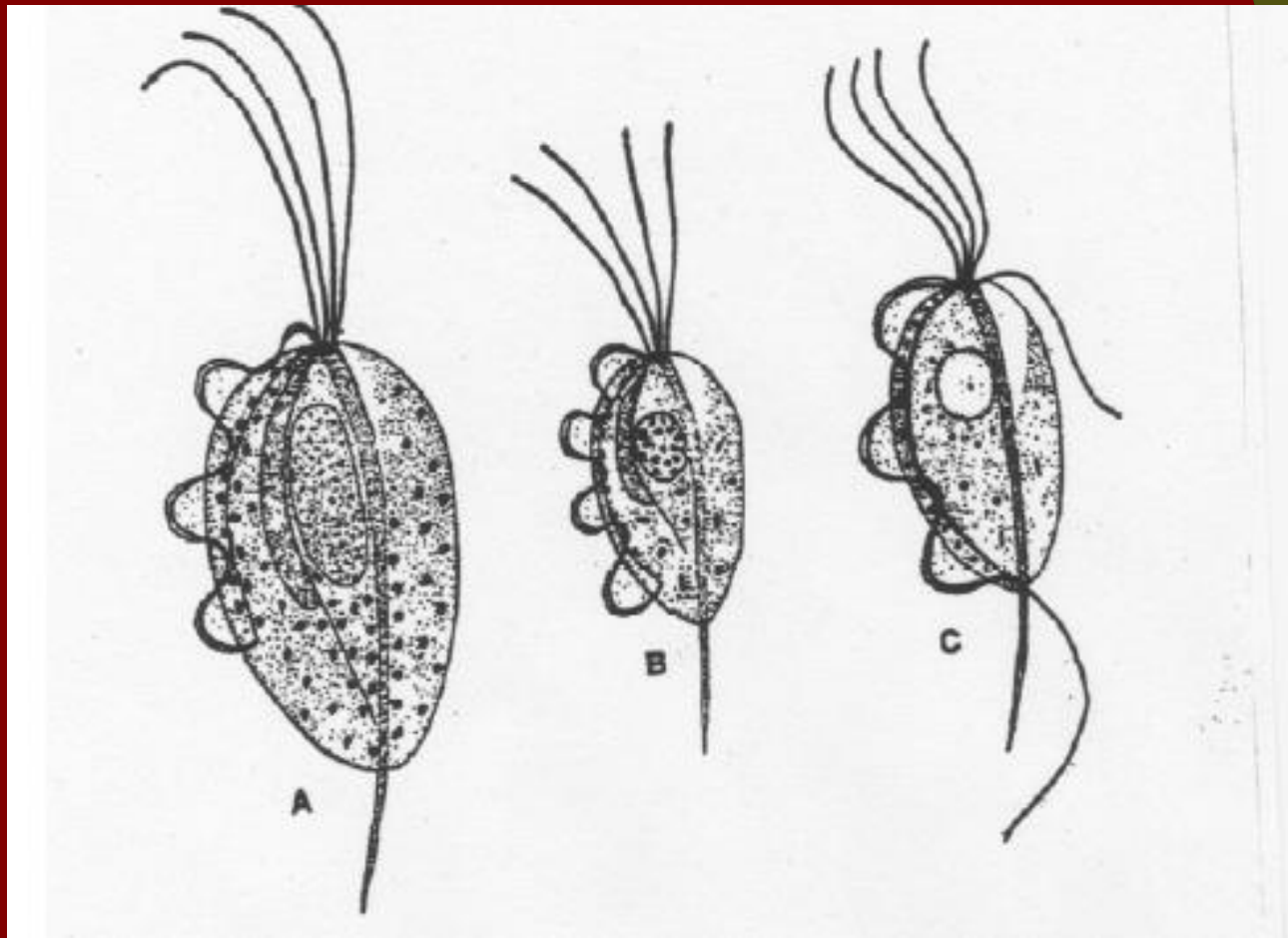


Trichomonas vaginalis



Trichomonas vaginalis

- ▶ one of most common STDs
 - ▶ ♀: feeds on mucosal surface of vagina
 - ▶ Yellow-green discharge, inflammation of genitals
 - ▶ ♂: urine, urethral discharge
 - ▶ Irritation, generally asymptomatic
- ▶ Urine centrifugation, light microscopy



A. *Trichomonas vaginalis*: 8 to 30 μm (Donne)

B. *Trichomonas tenax*: 6 to 17 μm (Muller)

C. *Trichomonas hominis*: 8 to 20 μm (Davaine)

Blood and Tissue Flagellates

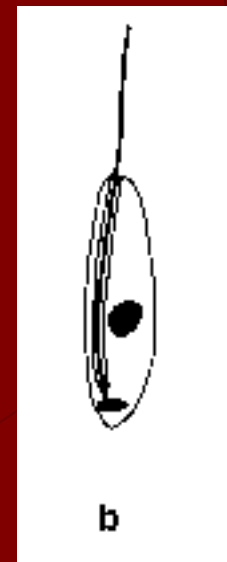
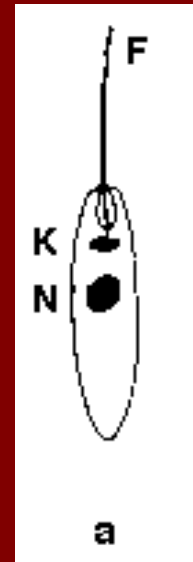
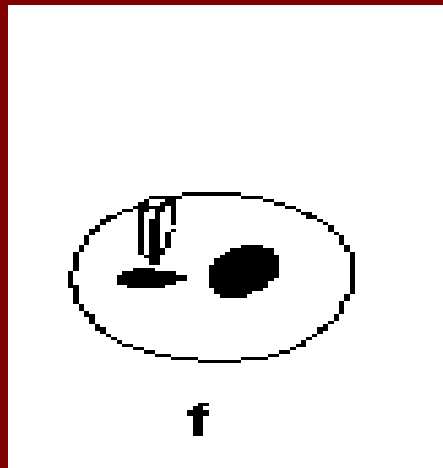
- ▶ Not diagnosed in the Philippines
 - ▶ *Trypanosoma* - *Triatoma*, *Rhodnius* bugs
 - ▶ *Leishmania* - *Phlebotomus*
 - ▶ Vectors are not found locally

*Monoxenous - with 1 hosts

*Heteroxenous - with 2 hosts

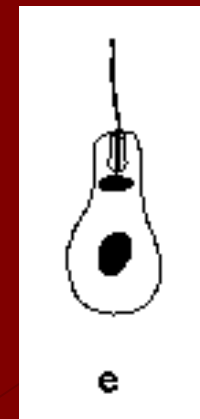
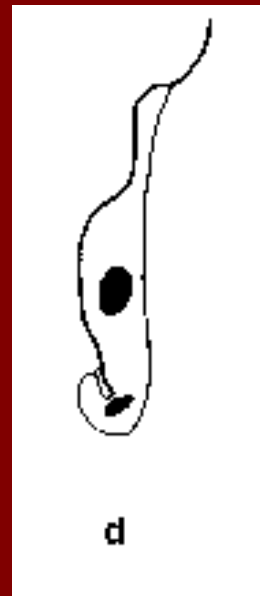
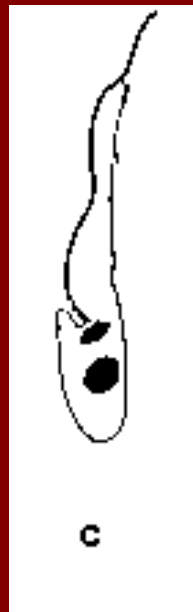
Blood and Tissue Flagellates

- ▶ Stages of development:
 - ▶ **Amastigote** - without flagellum
 - ▶ **Promastigote** - antenuclear kinetoplast
 - ▶ **Opisthomonastigote** - postnuclear kinetoplast



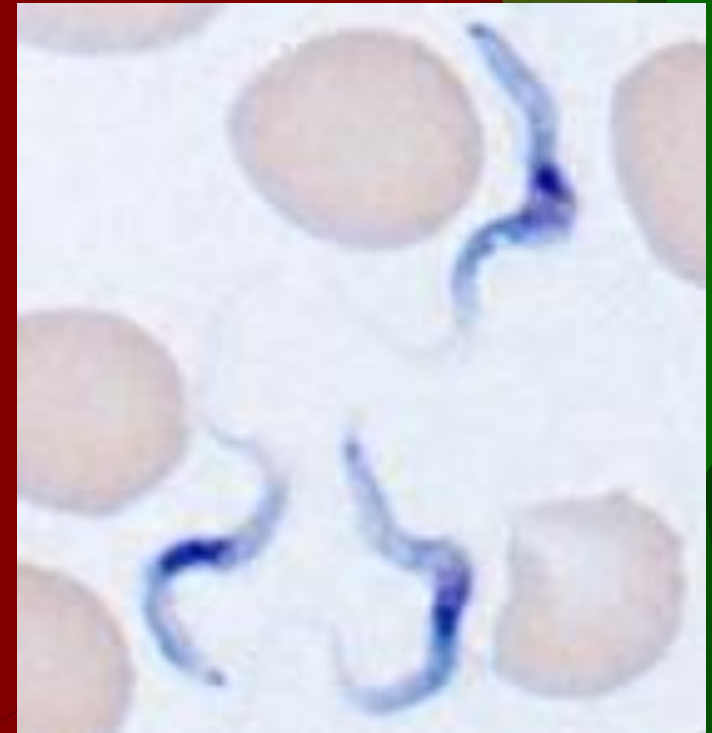
Blood and Tissue Flagellates

- ▶ **Epimastigote** - juxtannuclear kinetoplast
- ▶ **Trypomastigote** - postnuclear kinetoplast running along the undulating membrane
- ▶ **Choanomastigote** - antenuclear kinetoplast, funnel-shaped



Trypanosoma

- ▶ Hemoflagellates
- ▶ Spindle-shaped with nucleus
- ▶ Basal body or kinetosome - flagella arise
- ▶ Undulating membrane
- ▶ Salivaria
 - ▶ *T. brucei gambiense*
 - ▶ *T. brucei rhodesiense*
- ▶ Stercoraria
 - ▶ *T. cruzi*



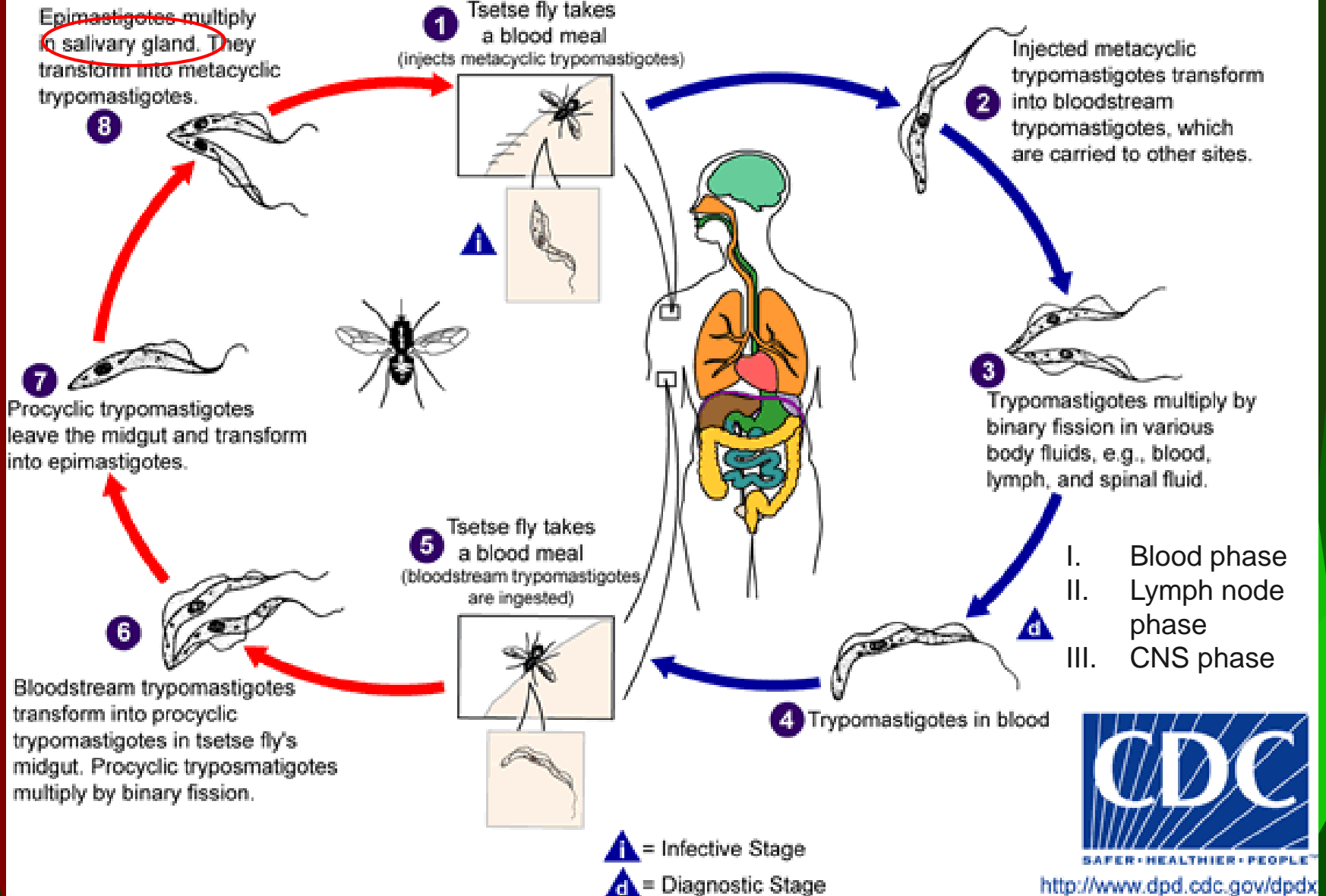
Salivarian

- ▶ Exhibits only **epimastigote** and **trypomastigote**
- ▶ 2 kinds of African Sleeping sickness
 - ▶ Gambian (chronic), Rhodesian (acute)
- ▶ 14-33 μm
- ▶ Vector: tsetse fly (*Glossina sp.*) - with cutting sponging mouthparts
 - ▶ *G. morsitans* (acute)
 - ▶ *G. palpalis* (chronic)



Tsetse fly Stages

Human Stages



Salivarian

- ▶ Multiplication → Remission → Relapse
- ▶ Remission
 - ▶ due to appearance of antibodies directed against the glycoprotein molecules coating the organisms
- ▶ Relapse
 - ▶ due to appearance of a small number of parasites expressing a **new version** of the glycoprotein coat (VSG)

Salivarian

▶ Pathology:

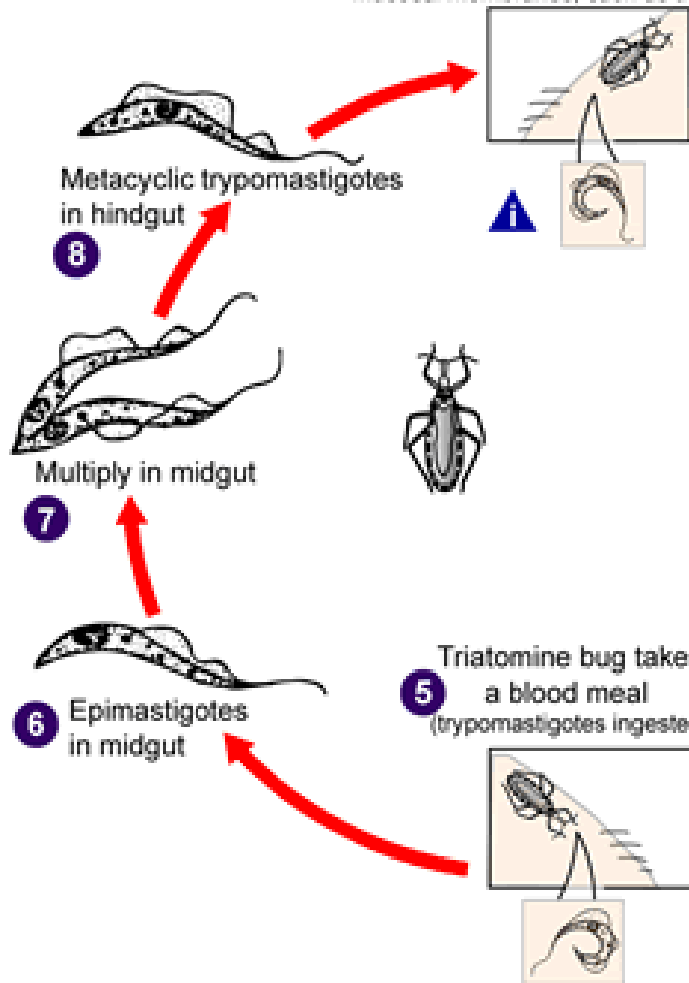
- ▶ Human African Trypanosomiasis (HAT)
- ▶ Earliest lesion - chancre develops at the site of the bite (18th d) resembles boil usu. painless
- ▶ Edema of the left eyelid (30th day)
- ▶ Trypanosomal rash (55th, 68th)
- ▶ *T. b. gambiense* - cervical lymphadenopathy - enlargement of lymph glands at neck (Winterbottom sign)
- ▶ w/o treatment -
 - ▶ invasion of the CNS (4-8mos *T.b.r.*; years *T.b.g.*)- headaches, abnormal behavior, leading to loss of consciousness and coma
→ Sleeping Sickness - death

Stercoraria

- ▶ Amastigote, trypomastigote, epimastigotes, promastigotes
- ▶ Chaga's disease
- ▶ vector: kissing bugs (*Triatoma*, *Rhodnius*, *Panstrongylus*)
- ▶ bloodstream trypomastigotes do not replicate (different from the African trypanosomes)

Triatomine Bug Stages

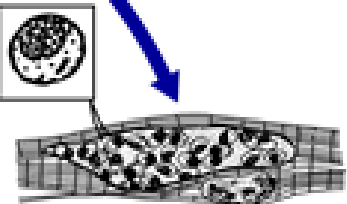
- 1** Triatomine bug takes a blood meal (passes metacyclic trypomastigotes in feces, trypomastigotes enter bite wound or mucosal membranes, such as the conjunctiva)



dx

Human 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.

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.

i = Infective Stage
d = Diagnostic Stage

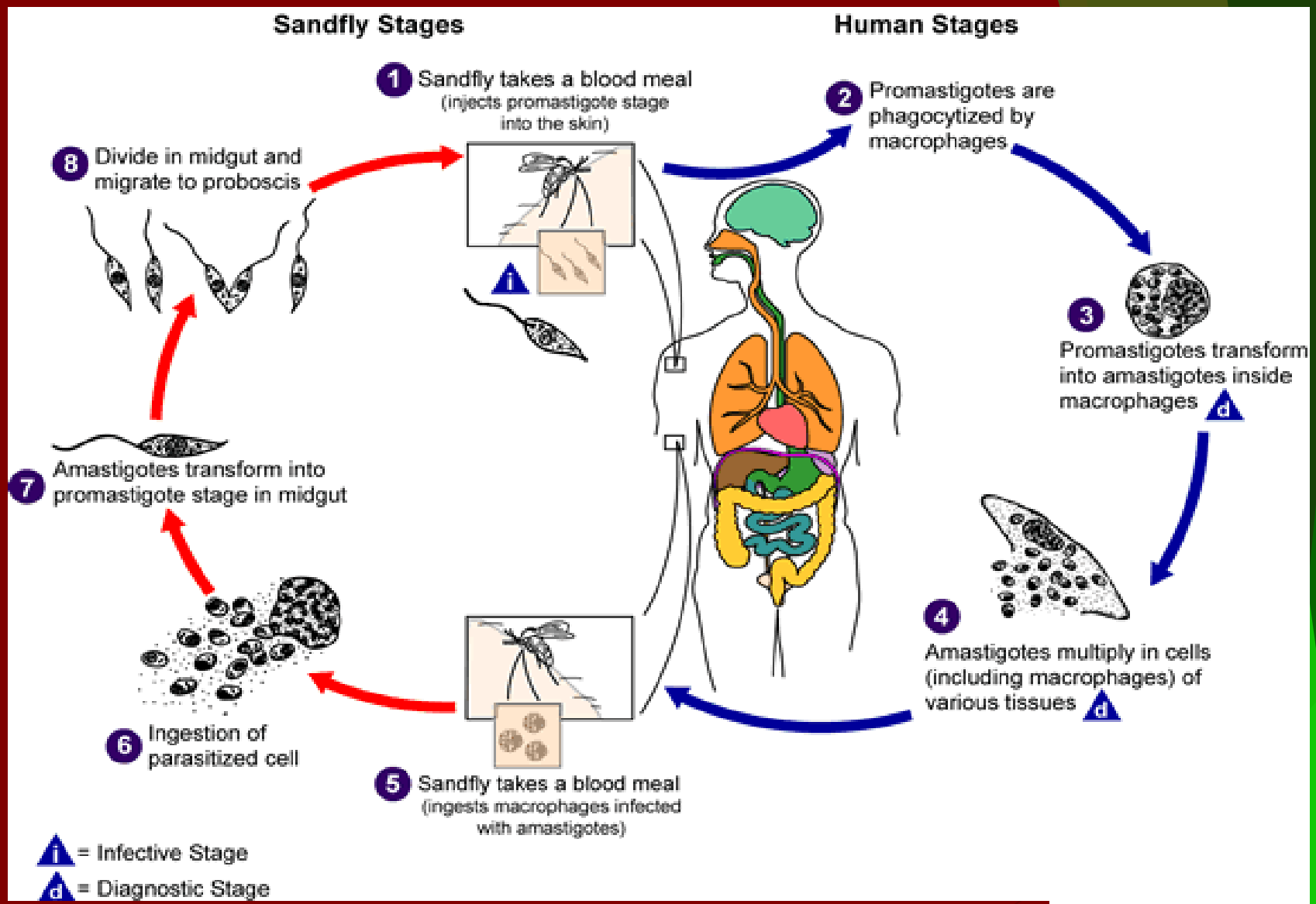
Stercoraria

▶ Pathology

- ▶ Chaga's disease: Romaas sign
- ▶ Acute - chronic stage may be asymptomatic
- ▶ Symptomatic chronic stage - may occur after years/decades
- ▶ Infection w/ heart - due to presence of amastigotes in ♥ -- cardiomyopathy
- ▶ Pathologies of digestive tract - megaesophagus, megastomach, megacolon

Leishmania

- ▶ Amastigote (mammalian host) and promastigote (sandfly) forms
- ▶ **Cutaneous leishmaniasis** - sores on skin where the sandfly fed, volcano w/ crater, erosion of skin
 - ▶ *L. tropica*
- ▶ **Mucocutaneous leishmaniasis**
 - ▶ *L. braziliensis*
- ▶ **Visceral leishmaniasis** - fever, weight loss, and an enlarged spleen and liver, low blood counts
 - ▶ *L. donovani*
- ▶ vector: sandfly (*Phlebotomus*)



- Amastigotes multiply in infected cells and affect different tissues, depending on the *Leishmania* species.

