AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 13.

Microbiology diagnosis of protozoan infections

FACULTY: General Medicine
SUBJECT: Medical microbiology - 2

Discussed questions:

1. Classification and general characteristics of protozoa.
2. Classification of protozoa:

Sarcomastigophora type:

a) Subtype Sarcodina:

• Entamoeba hystolitica, morpho-biological characteristics, pathogenesis, microbiological diagnosis of amebiasis (microscopic, histological, parasitological, serological methods)

b) Subtype Mastigophora:

• Giardia lamblia, morpho-biological characteristics, pathogenesis, microbiological diagnosis

• Morpho-biological characteristics, pathogenesis, microbiological diagnosis of Trichomonas genus (T.vaginalis).

• Leischmania genus (L.donovani, L.tropica), morpho-biological characteristics, pathogenesis, microbiological diagnosis (microscopic, parasitological, serological methods)

• Causes of trypanosomosis (T. brucei, T. cruzi), morpho-biological characteristics, pathogenesis, microbiological diagnosis

3. Type Apicomplexa:

• Plasmodium genus (P.malariae, P.vivax, P.ovale, P.falciparum), morpho-biological characteristics and life cycle. Pathogenesis of the disease. Microbiological diagnostics (microscopic, serological, express method)

• Toxoplasma gondii, morpho-biological characteristics, pathogenesis, microbiological diagnosis (microscopic, parasitological, serological (IFA, IFR, KBR, PHAR), skin-allergic methods)

•*Cryptosporidium*, morpho-biological characteristics, pathogenesis, microbiological diagnosis

•*Blastocystis hominis*, morpho-biological characteristics, pathogenesis, microbiological diagnosis

4. Ciliophora type:

• Balantidium coli, morpho-biological characteristics, pathogenesis, microbiological diagnosis

Microspora type, Microsporidium genus – as an obligate intracellular parasite

Purpose of the lesson:

- To acquaint students with the morpho-biological characteristics of pathogenic protozoa, to teach them laboratory diagnostics of the causative agents of amebiasis, giardiasis, trichomoniasis, balantidiosis, malaria, toxoplasmosis, leishmaniasis and trypanosomosis.

❑List of Intestinal Parasites

Sarcodina:	***Entamoeba histolytica*** **
	Entamoeba dispar
	Iodomoeba butschlii
	Endolimax nana
	Entamoeba coli
	Entamoeba hartmani
	Dientamoeba fragilis **
Apicomplexa:	***Cryptosporidium parvum.*** **
	Isospora belli **
	Cyclospora cayetanensis **

(**=pathogenic)

Cont…

Mastigophora:	**Giardia lamblia****
	Trichomomas hominis
	Chilomastix mesnili
Ciliophora:	**Balantidium coli****
Microsporidia:	**Enterocytozoon bienusi****

(**=pathogenic)

INTRODUCTION

kingdom : Protista
sub kingdom: protozoa
Phylum: Sarcomastigophora
Sub phylum: Sarcodina
Class: Lobosea
Sub class: Gymnamoebia
Order: Amoebida
Sub order: Tubulina
Genus: Entamoeba
Species: histolytica

- **Amoeba**
  - **Free living**
  - **Intestinal**
- Entamoeba histolytica is an intestinal amoeba
- All intestinal amoebae are non pathogenic except Entamoeba histolytica
- All free living amoeba are oppurtunistic pathogens.

Entamoeba hisyolytica

- Amoeba are structurally simple protozoans which have no fixed shape
- Phylum : Sarcomastigophora
- Subphylum :Sarcodina
- Super class :Rhizopoda
- Order : Amoebida

MORPHOLOGY

The morphology of E. histolytica shows three different stages.

1. Trophozoite (the growing or feeding stage).
2. Pre- cystic stage.
3. Cystic stage.

Trophozoite:

Shape: not fixed because of constantly changing position.

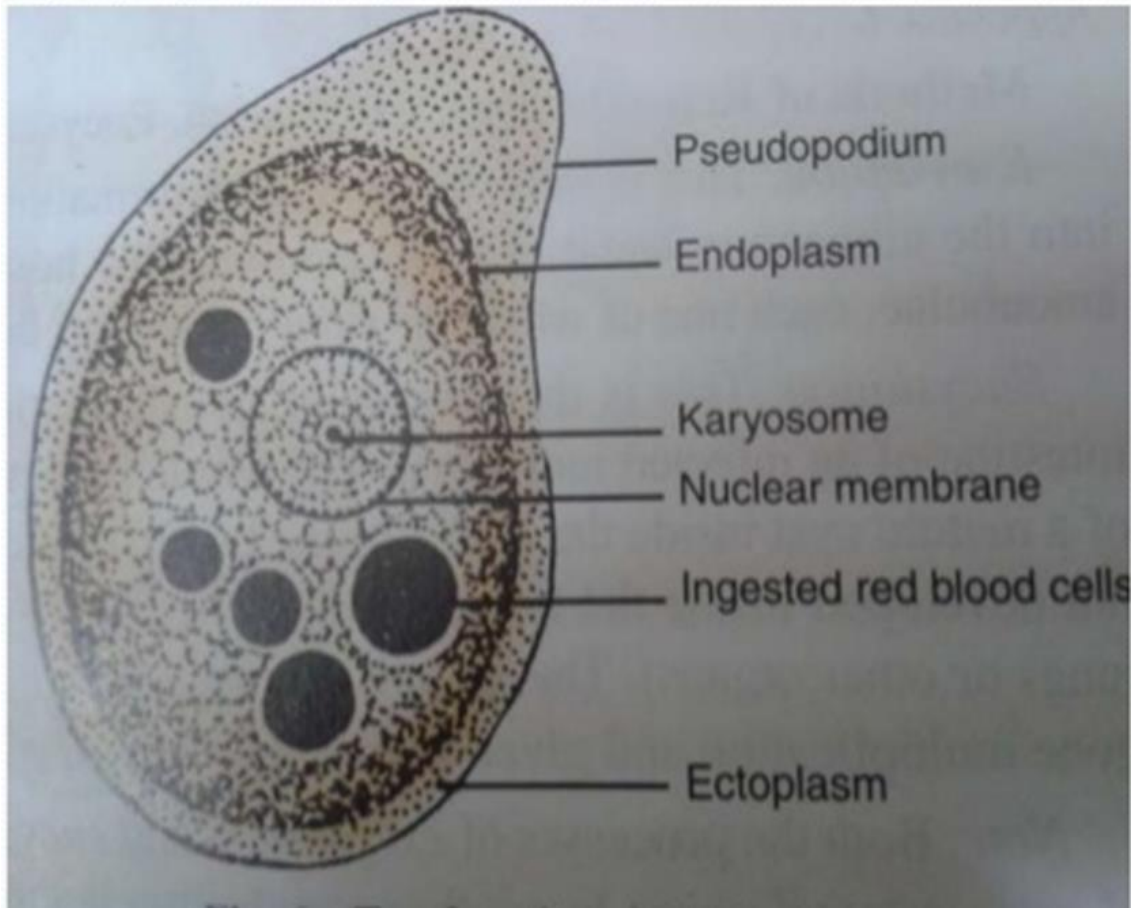
Size: Average being 20-30 um.

Cytoplasm: divisible into two portion,

- a clear translucent ectoplasm.
- a granular endoplasm.

- Red blood cells, occasionally leucocytes and tissue debris are found inside the endoplasm.

TROPHOZOITE



Pre-cystic stage

Size – 10-20um

shape – round or slightly ovoid with a blunt pseudopodium projecting from the periphery.

Endoplasm is free of red blood cells and other food particles.

Nuclear structure is same as that of trophozoite.

Cystic stage

The cyst varies greatly in size:

- the small race being 6to9um.
- the large race being 12-15um.

During encystment, the parasite becomes rounded and is surrounded by a highly refractile little membrane, called the cyst wall.

A mature cyst is a quqdrinucleate spherical body.

The cyst begins as a uninucleate body but soon divides by binary fission and develops into binucleate and quadrinucleate bodies.

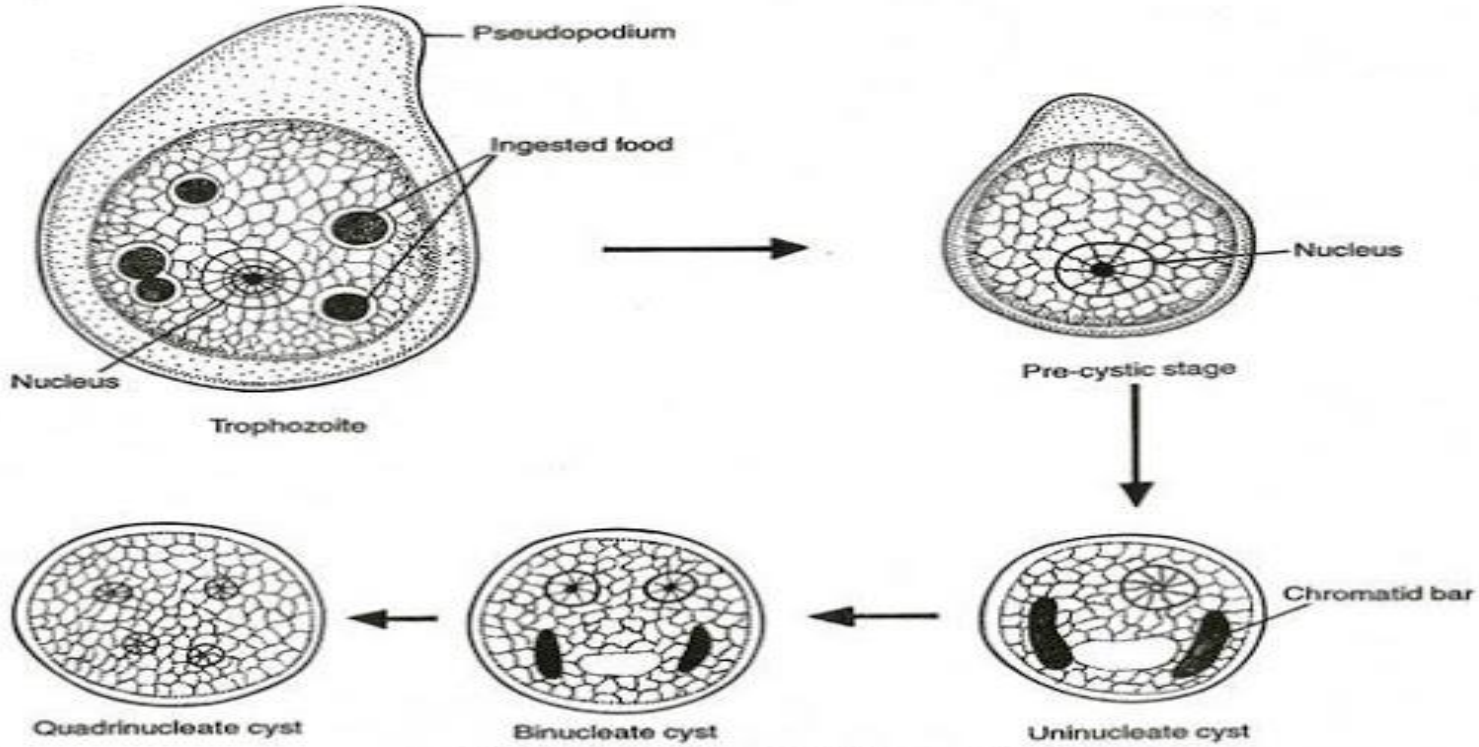
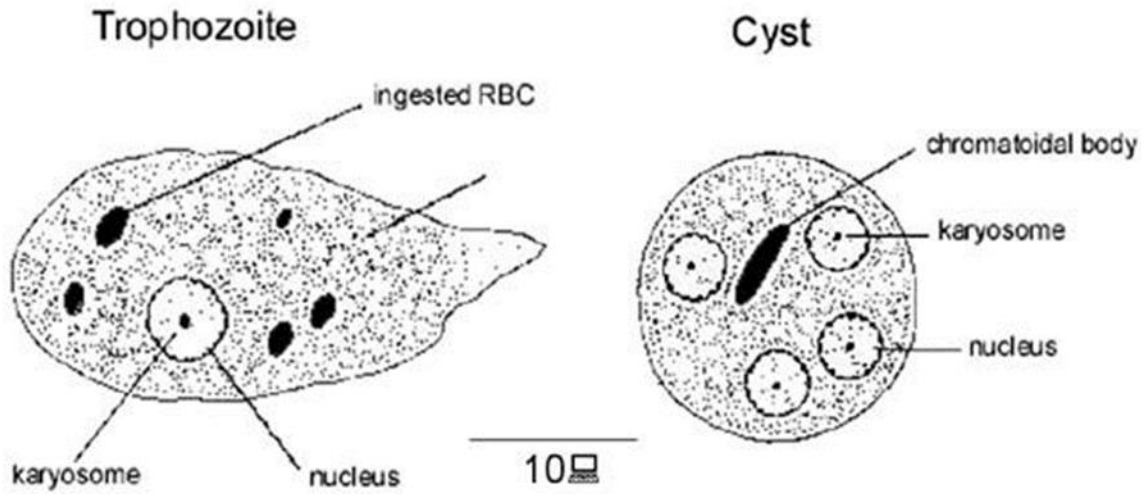


Fig. 174. Stages of life cycle of *Entamoeba histolytica*.



LIFE CYCLE

- Infective form :mature quadrinucleate cyst passed in feces of convalscents and carriers
- Mode of transmission :man acquires infection by swallowing food and water contaminated with cyst .
- Stomach –cyst wall is resistant to gastric juice
- Exystation :cyst reaches the caecum or lower part of ileum ,due to alkaline medium ,cyst wall damaged by trypsin ,leading to exystation

PATHOGENESIS AND CLINICAL FEATURES

- E.histolytica causes **intestinal** and **extra intestinal** amoebiasis

  Intestinal amoebiasis -**PATHOGENESIS**

  Lumen *dwelling amoeba do not cause any illness .They causes disease only when they invade the intestinal tissues .*

  10 % -symptomatic

  90% -asymptomatic

Pathogenicity

- **Incubation period**: 4-5 days.
- **Clinical features or Symptomatology**: The term amoebiasis is used to denote all those condition which are produce in the human host by infection with E. histolytica.
- **Amoebic dysentry**: is a condition in which the infection is confined to the intestinal canal and is characterised by the passage of blood and mucus in the stool.

Specimen:

The various sample collected for laboratory investigation include **stool, swabs, aspirated pus, blood, CSF, biopsied and autopsied material.**

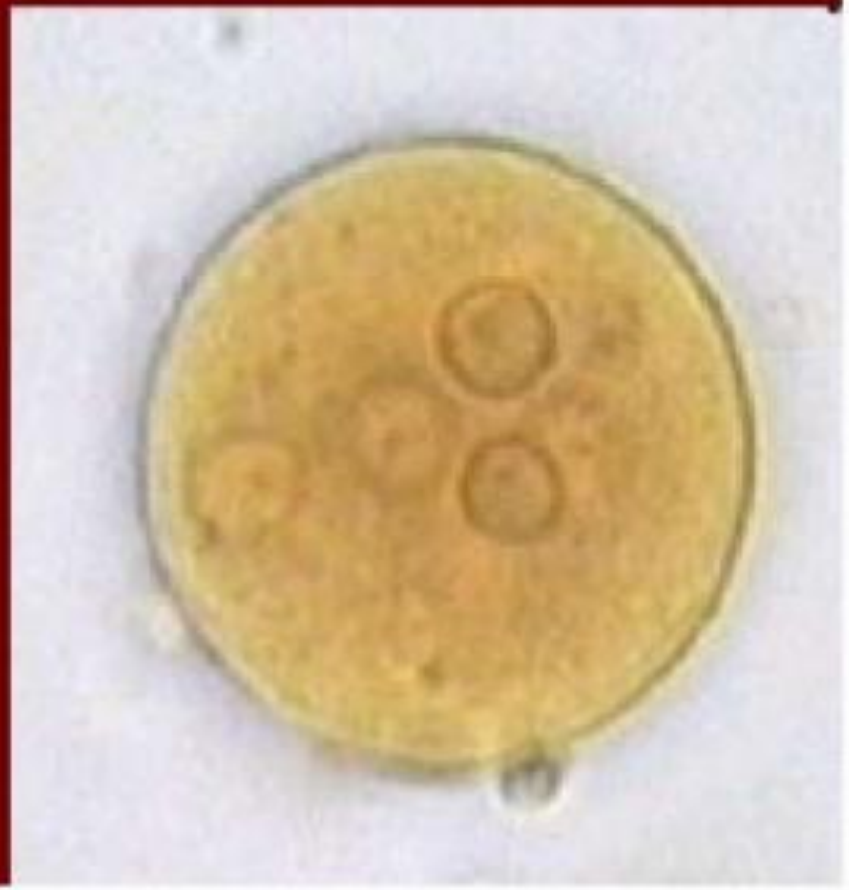
1. Examination of Stool:

a) Naked eye or Macroscopic appearance:

- An offensive dark brown semi-fluid stool
- Acid in reaction
- Admixed with blood and mucus.

Microscopic examination of Stool

- A sample of freshly collected fecal specimen containing mucous and blood is transferred on a slightly warm slide and covered with cover slip and examined microscopically
- Doctortvrao's 'e' learning series



2. Examination of Blood:

- Shows moderate leucocytosis.

3. Serological Test:

- In early cases it is always negative.

ELISA, Indirect haemagglutination, Dot ELISA, latex agglutination test are in use.

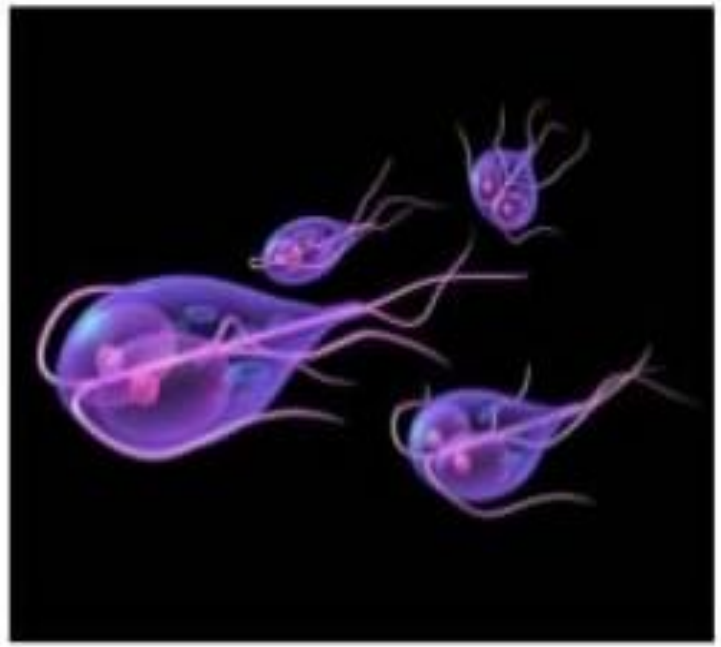
CULTURE

- **Cultures are not done routinely.**

Treating Amebiasis.

- Frequently, either **metronidazole** (Flagyl) or **tinidazole** (Fasigyn) are used to treat Amebiasis. If this does not work, **Chloroquine,** emetine, and dehydroemetine can be used. Eliminating cysts in carriers who do not have symptoms is accomplished with **diloxanide furoate (Furamide), iodoquinol (Yodoxin**), and **paromomycin. Nitazoxanide** is a newer drug that shows promise against not only *E. histolytica* but many other parasites as well.

GIARDIA



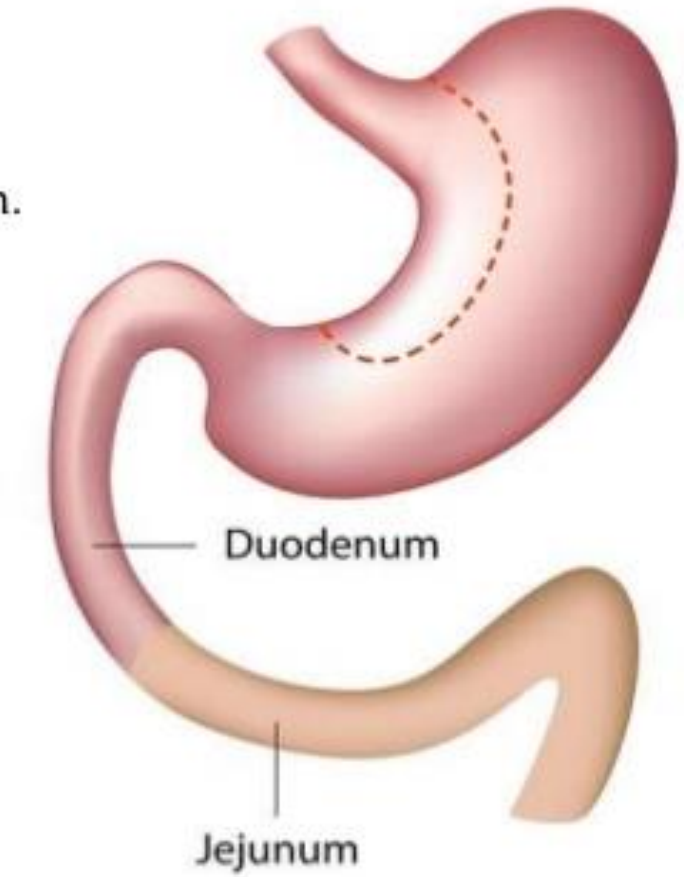
INTRODUCTION

- Phylum: SARCOMASTIGOPHORA
- Subphylum: MASTIGOPHORA
- Class: ZOOMASTIGOPHORA

- The parasites belonging to this group possess one or more **whip-like flagella**.
- So they are known as flagellates.

HABITAT

- Duodenum & the upper part of the jejunum.
- THE ONLY PROTOZOAN PARASITE FOUND IN THE LUMEN OF HUMAN SMALL INTESTINE.



MORPHOLOGY

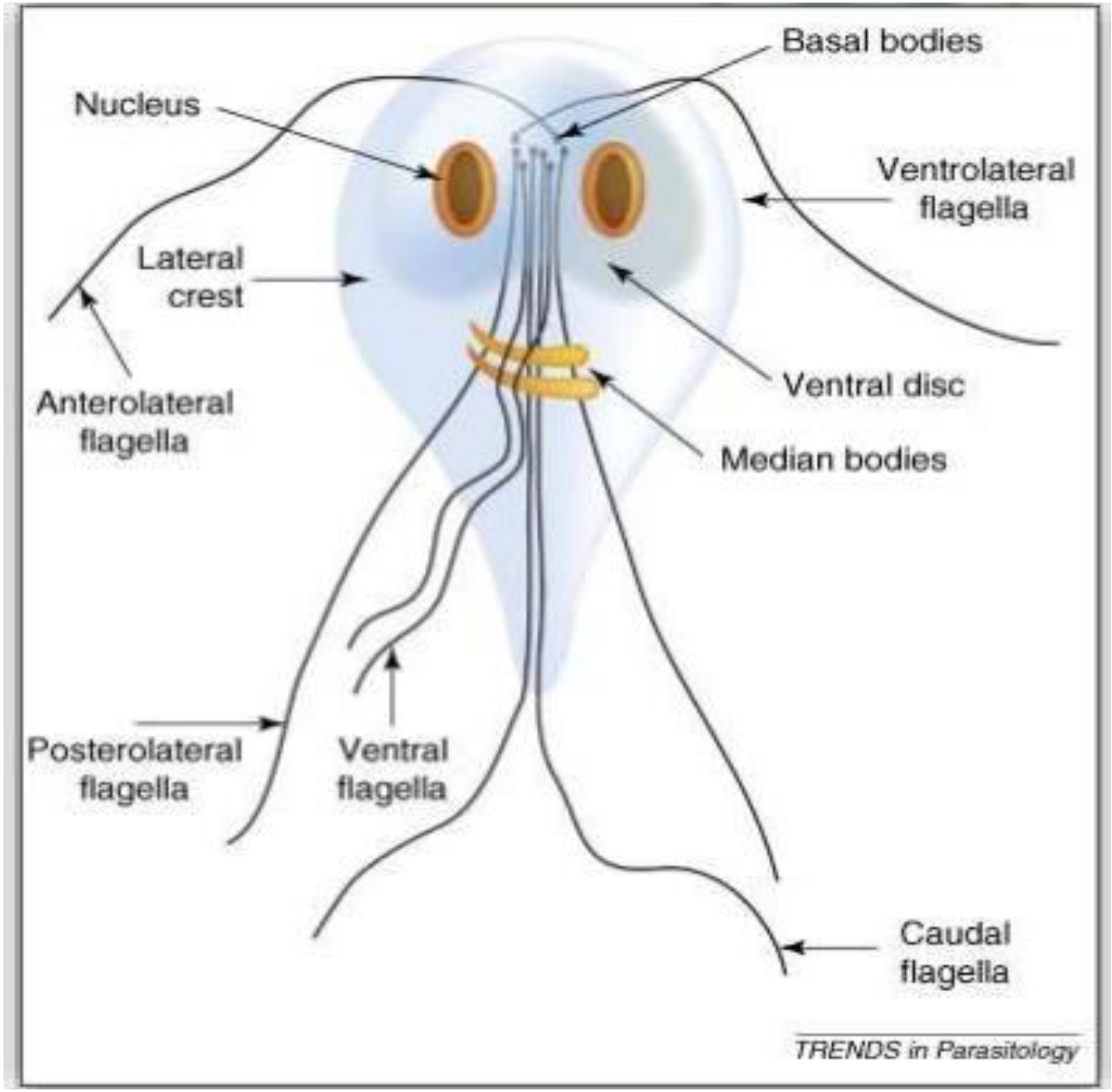
- It exists in two forms –

1) Trophozoit (Vegetative form)

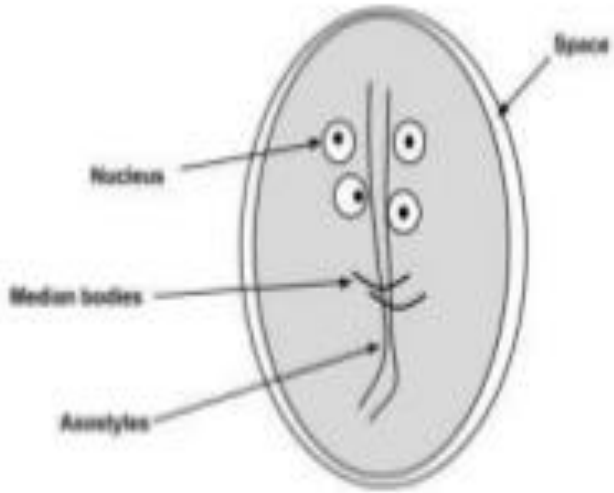
2) Cyst (Infective form)

TROPHOZOITE

- Tennis racket or heart shaped or pyriform shaped.
- Dorsal surface – convex
- Ventral surface – concave & having sucking disk (for attachment)
- 14 μm x 7μm x 4μm
- Anterior end – broad & rounded
- Posterior end – tappers to a sharp point
- Bilaterally symmetrical :
  - Nuclei – 1 pair
  - Flagella with blepharoblast – 4 pair
  - Axostyle – 1 pair (along the midline)
  - Parabasal / Median body – 1 pair (transverse & posterior to sucking disc)
- Falling leaf motility around its long axis.


Basal bodies
Nucleus
Ventrolateral flagella
Lateral crest
Anterolateral flagella
Ventral disc
Median bodies
Posterolateral flagella
Ventral flagella
Caudal flagella
TRENDS in Parasitology

CYST



- Round or oval in shape.
- Surrounded by hyaline cyst wall.
- 12μm x 7μm.
- Axostyle – diagonally placed, form a deviding line within cyst.
- 4 nuclei – clustered at one end or at opposite poles (each pairs).
- Remnants of flagella and margins of the sucking disc may be seen inside the cytoplasm of a young cyst.
- An acid environment often causes the parasite to encyst.

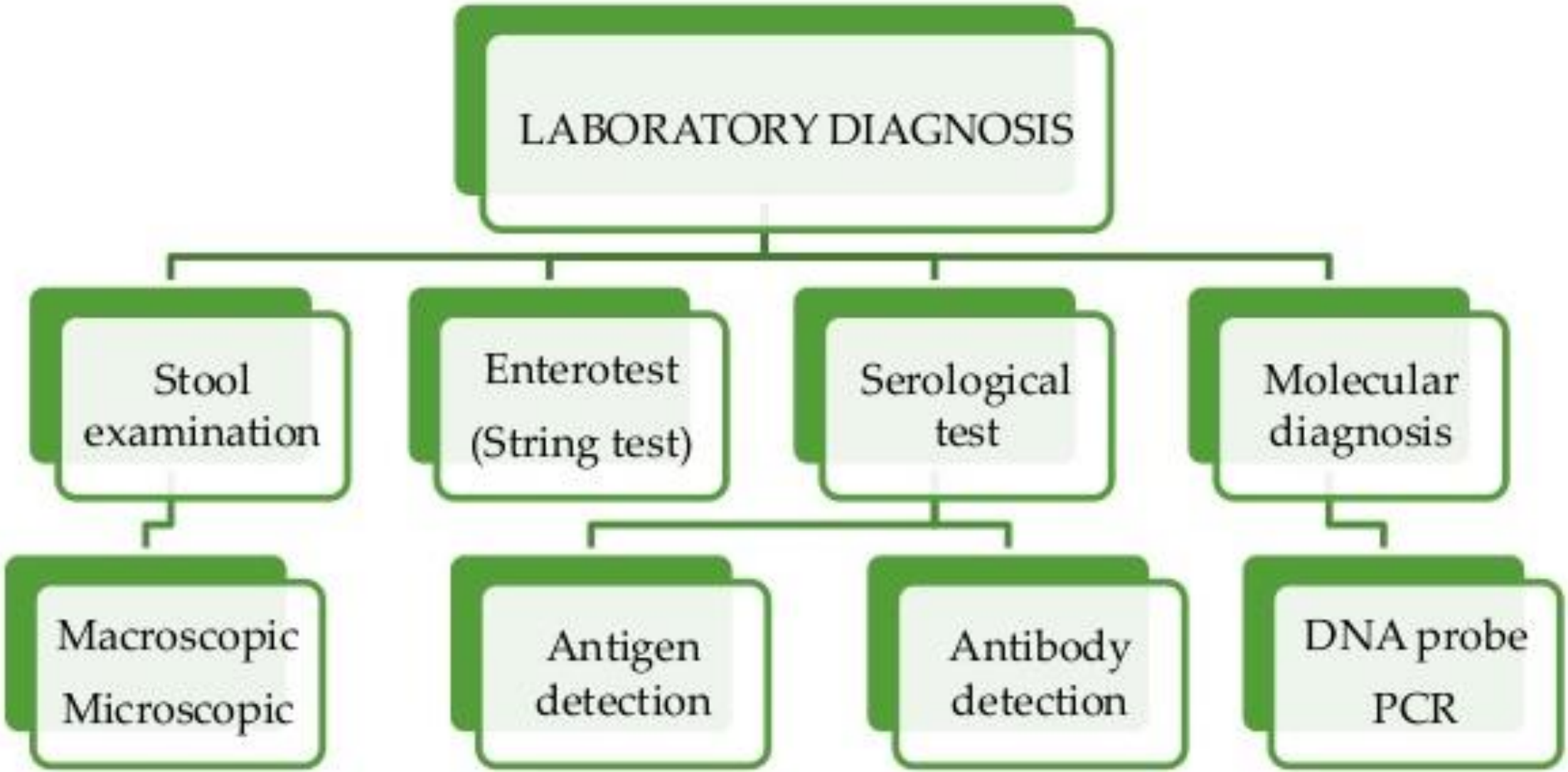
MODE OF TRANSMISSION

- Infection is occured by ingestion of cyst in contaminated food & water.
- Direct transmission from person to person may occure in children, male homosexuals, mentally ill persons.

CLINICAL FEATURES

1. Silent cases without any symptoms.
2. Intestinal :
    1. Malabsorption syndrome (Steatorrhoea)
    2. Mucus diarrhea
    3. Dull epigastric pain
    4. Flatulence
    5. Chronic enteritis
    6. Acute enterocolitis
3. General :
    1. Fever
    2. Anaemia
    3. Weight loss
    4. Allergic manifestations.
4. Chronic cholecystopathy.

Incubation period : about 2 weeks


LABORATORY DIAGNOSIS
Stool examination
Enterotest (String test)
Serological test
Molecular diagnosis
Macroscopic
Microscopic
Antigen detection
Antibody detection
DNA probe
PCR

STOOL EXAMINATION

- Identification of cysts in formed stool and trophozoites & cysts in diarrhoeic stool or after a purgative.
- In asymptomatic carriers only cysts are seen.
- Macroscopy : offensive odour, pale coloured & fatty stool.
- Microscopy : salaine & iodine wet preparations.
- Multiple specimens need to be examined.
- Concentration techniques like formal ether or zinc acetate are used.

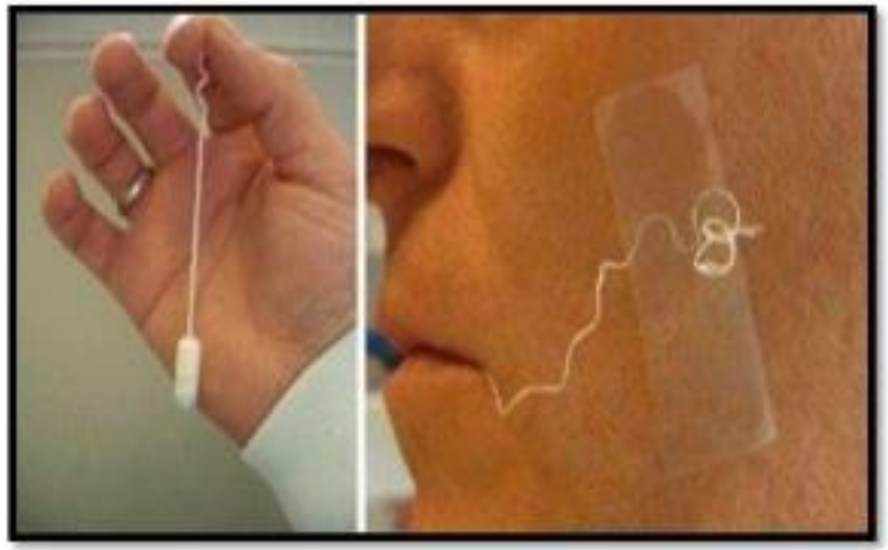
ENTEROTEST (STRING TEST)

- Method for obtaining duodenal specimen (upper part of small intestine)
- Procedure :
  - A coiled string with a small weighted gelatin capsule is swallowed by the patient & the free end of the string is attached to the side of the patient's face.
  - The capsule dissolves in the stomach & the string which is weighted at its distal end, passes into the duodenum.
  - After 2-4 hrs the string is withdrawn & placed in a saline with mechanical shaking.
  - The centrifuged deposit of saline is examined by wet mount technique to detect the presence of motility of the organism or specific morphological forms of trophozoites of Giardia (and larvae of Strongyloides stercoralis).

When the test should performed

- Entero-test is performed when a physician suspects a parasitic infection, but no parasites were found in stool sample.

- As its sensitivity is comparable to duodenal aspirate, it eliminates the need of duodenal intubation.



SERODIAGNOSIS

- Antigen detection in feces –
  - ELISA
  - IIF (Indirect immunofluroscent tests)
  - Immuno-chromatographic strip test

- Antibody detection –
  - IIF
  - ELISA

MOLECULAR METHOD

- DNA based techniques are available now.
- They are used to demonstrate the genome of the parasite.
  - PCR
  - DNA probe

TREATMENT

- Metronidazole – 250mg x 3 times daily x 5 days. **(Cure rate -95%)**
- Tinidazole – 2 gm single dose. **(More effective)**
- Furazolidone
- Nitazoxamide

→ **Children (less adverse effects)**

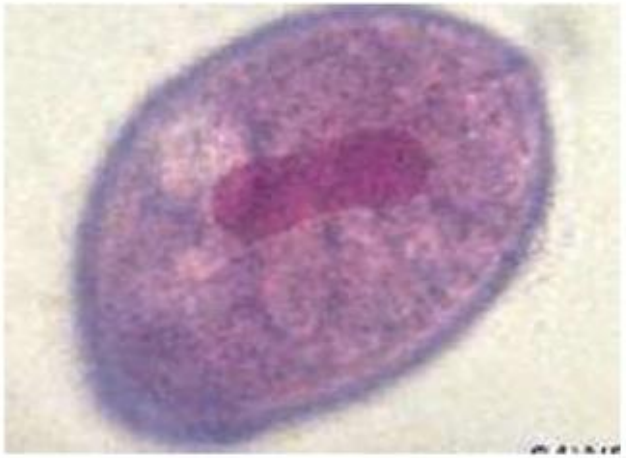
- Parmomycin → **Pregnant female**

INTRODUCTION

- It is largest protozoan
- Only ciliated parasites of humans
- Causes **Balantidiasis**

Taxonomy:
belongs to Phylum Ciliophora
Class: Litostomatea
Order: Vestibuliferida
Family: Balantidiidae

Balantidium coli



Habitat: large intestines of man, pig (main reservoir) and other animals.

MORPHOLOGY

- Two forms:

a. Trophozoite – in dysenteric stool

b. Cyst: - in carriers and chronic cases

Both forms: binucleated - large macronucleus and small micronucleus

TROPHOZOITE

- Found in active stage of disease – invasive form
- shape: oval
- Size: 30-300 µm long x 30-100 µm breadth
- Whole body covered with a row of tiny delicate cilia – organ of locomotion
- Cilia present near the mouth part – longer → called "**adoral cilia**"
- Anterior end- narrow

- Bears a groove (**peristome**) that leads to a mouth (**cytostome**)
- followed by a short funnel shaped gullet (**cytopharynx**) extending up to one-third of the body.

- Posterior end- broad, round

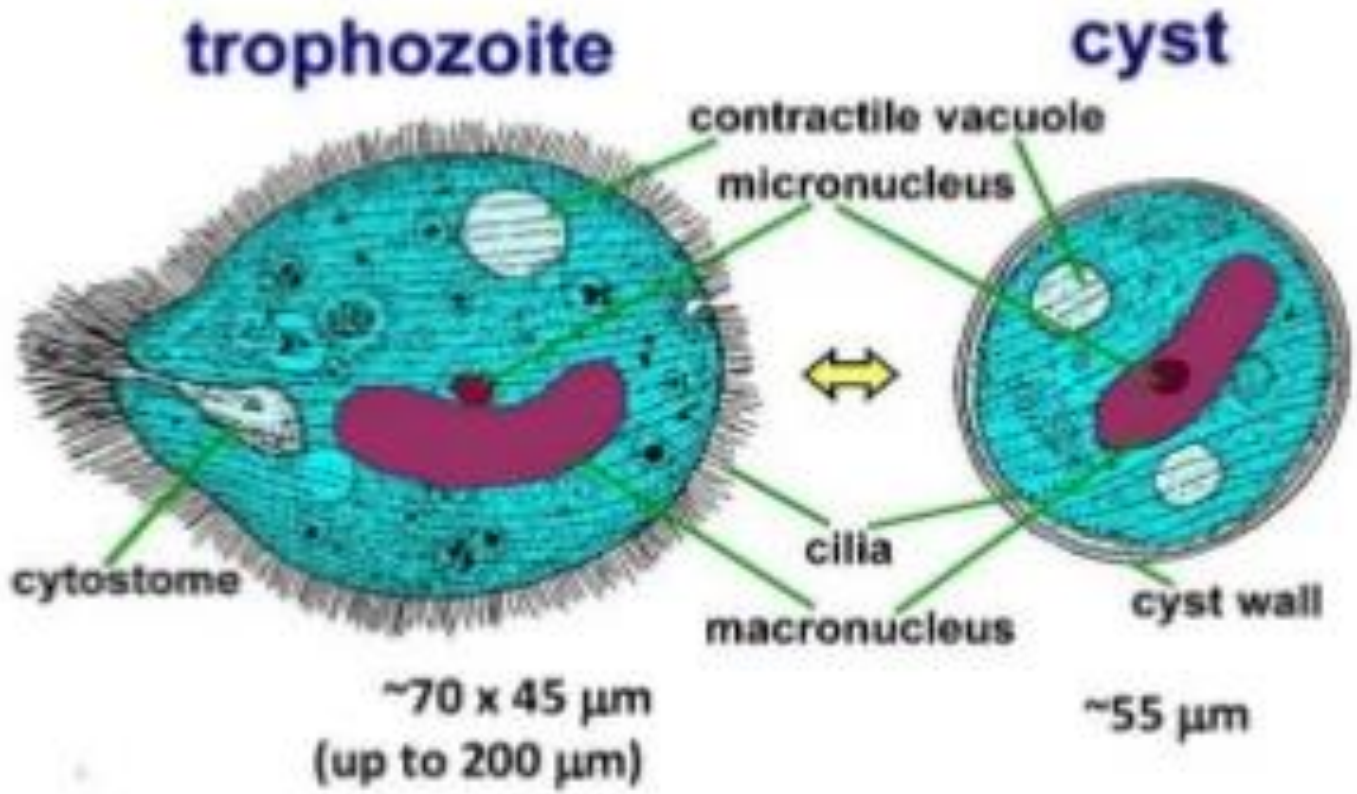
- Bears an excretory opening (**cytopyge**)

CYST

- Shape: round
- Size: 40-60 µm
- Immobile and dominant
- Surrounded by a thick transparent cyst wall→ allows the cysts to resist degradation in the acidic environment of the stomach and the basic environment of the small intestine

- Contains two nuclei- macronucleus and micronucleus and vacuoles
- Cilia- seen in younger cyst but is absorbed on maturity→ movement ceases

Balantidium coli



Development in large intestine- Life cycle

- Mode of transmission: faecal-oral route
- Virulence factor: Hyaluronidase- help to penetrate intestinal mucosa
- Excystation: occurs in small intestine- when trophozoites are produced from cysts

- Multiplication in large intestine
- Single trophozoite forms from each cyst
- trophozoite- is the feeding stage of the parasite → multiply either in gut lumen or enter the sub mucosa of large intestine

Cell division → Asexual reproduction
Cell division → Sexual reproduction

Clinical features

Asymptomatic carriers

- Results from majority of infection
- Harbours the cyst and spread the infection

Acute disease

- Similar to acute amoebic dysentery
- Trophozoites invade gut sub mucosa- form multiple tiny superficial ulcers

- Ulcers with necrotic base and undermined edge

- Microscopically- cluster of trophozoites are found in sub mucosa with inflammatory cells (lymphocytic)
- Patients present have frequent diarrhoea with profuse mucus and blood.
- Other features- fever, nausea, vomiting and abdominal pain
- Haemorrhage- may lead to shock and death

LABORATORY DIAGNOSIS

- Stool examination-detects trophozoites and cysts
- Histopathology
- Culture
- serology

STOOL MICROSCOPY

- Trophozoites- detected in acute disease (dysenteric stool)

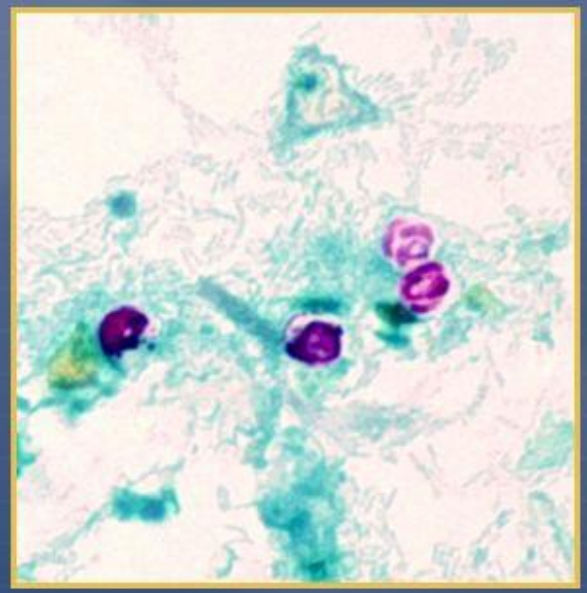
-easy to identify by its rotatory motility, large kidney shaped macronucleus and presence of cilia

- Cysts- seen in chronic cases or carriers

- round, 40-60 µm in size, surrounded by a cyst wall and presence of two nuclei

Cryptosporidium parvum

- Coccidian protozoa
- Obligate intracellular pathogen
- Primarily infects intestine
- Forms oocysts
  - Resistant to disinfection
  - Killed by ozone, desiccation



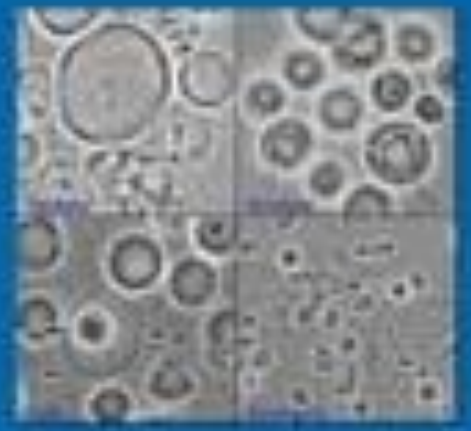
Oocysts

- Oocysts are 4-5 microns
- Oocysts survive extreme conditions
  - Oocysts infective for 2-6 months in environment

- Oocysts in stool appear with onset of symptoms
- Oocysts are immediately infectious
  - Infection may result from 10 oocysts
- Oocysts shed for several weeks after symptoms resolve
- Asymptomatic infections appear to be common

Mode of Transmission

- Fecal-oral
- Waterborne
- Foodborne
- Community
  - Person to person, esp. child care settings
- Hospital
  - Patients to health care staff, patient-to-patient
- Aerosol infection



Life Cycle

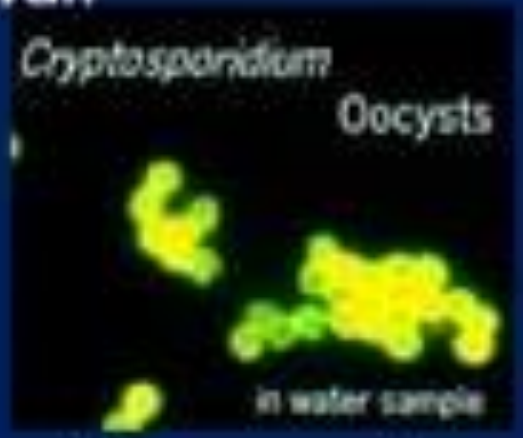
- Excystation -release of the four sporozoites
- Invasion of intestinal epithelial cells
- Asexual life cycle
- Sexual life cycle
  - Differentiation of micro and macrogametes
- Development of oocysts
- Formation of new, infectious sporozoites

Symptoms

- In immunocompetent patients, include :
  - Frequent, watery diarrhea (1-2 week duration), yet self-limiting
  - Nausea, Vomiting , Abdominal cramps, and Low-grade fever
- For immunocompromised persons, the illness is more severe:
  - Debilitating, cholera-like diarrhea (up to 20 liters/day), electrolyte imbalance, dehydration and sometimes death.
  - Severe abdominal cramps, Malaise, Low-grade fever, Anorexia and Weight loss.

Diagnosis

- Microscopy with an acid fast stained stool smear, which will stain the oocysts bright red.
  - Another form of microscopy is fluorescent microscopy using monoclonal antibody to oocyst wall



Direct immunofluorescence antibody stain

acid-fast oocyst stain

Diagnosis

- Enzyme immunoassay (ELISA), for the detection of cryptosporidial antigens in stool samples, has greatest sensitivity and specificity
- Molecular methods using PCR

Treatment

- Nitazoxanide
  - Interferes with folate production
  - Prevents parasite replication
- Immunocompetent
  - *C. parvum* will usually pass on its own
- Immunocompromised
  - AIDS patients: treat with antiretrovirals and strengthen immune system, no cure
  - Others: would not benefit from antiretrovirals; keep hydrated

Microsporidiosis

- *[Anncaliia spp.] [Encephalitozoon cuniculi] [Encephalitozoon hellem] [Encephalitozoon intestinalis (syn. Septata intestinalis)] [Tubulinosema acridophagus] [Enterocytozoon bieneusi] [Nosema spp.] [Pleistophora sp.] [Trachipleistophora spp.] [Vittaforma corneae (syn. Nosema corneum)]*

Microsporidiosis

- The microsporidia are a group of unicellular intracellular parasites closely related to fungi, although the nature of the relation to the kingdom Fungi is not clear. The taxonomic position of this group has been debated and revised repeatedly; historically, they were considered protozoa and often remain managed by diagnostic parasitology laboratories. Microsporidia are characterized by the production of resistant spores that vary in size (usually 1—4 µm for medically-important species). They possess a unique organelle, the polar tubule or polar filament, which is coiled inside the spore as demonstrated by its ultrastructure. Microsporidia also possess degenerated mitochondria called mitosomes and lack a conventional Golgi apparatus.

Microsporidiosis

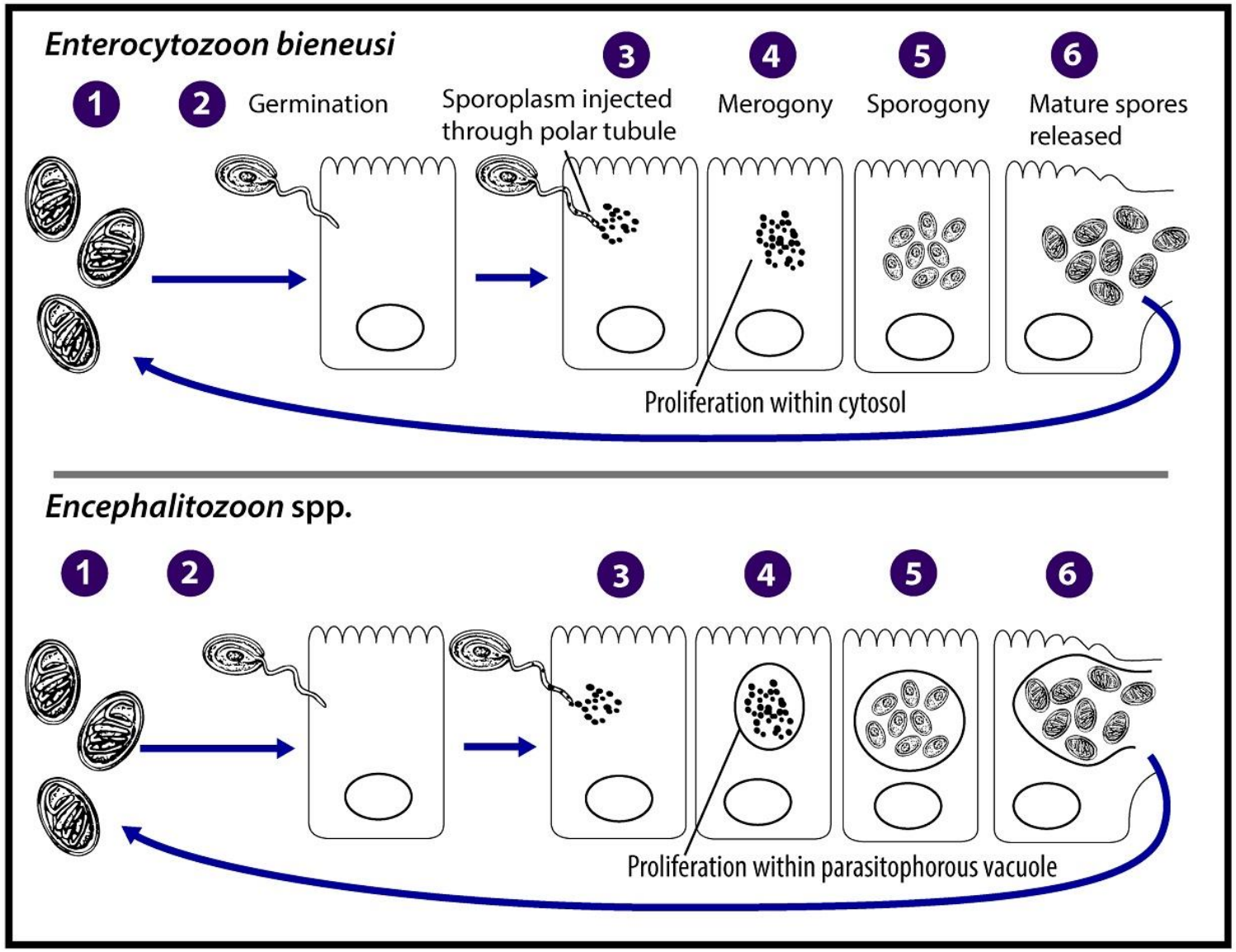
- To date, more than 1400 species belonging to over 200 genera have been described as parasites infecting a wide range of vertebrate and invertebrate hosts. There are at least 15 microsporidian species that have been identified as human pathogens; the vast majority of cases being caused by ***Enterocytozoon bieneusi,*** followed by some ***Encephalitozoon*** species (*E. cuniculi, E. hellem*, *E. intestinalis* (=*Septata intestinalis*)). Other less frequently reported agents include members of the genera ***Anncaliia*** (=*Brachiola*) (*A. algerae*, *A. connori, A. vesicularum*), ***Microsporidium*** (*M. ceylonensis*, *M. africanum*), ***Trachipleistophora*** (*T. hominis, T. anthropophthera*), ***Nosema ocularum***, ***Pleistophora ronneafiei***, ***Vittaforma corneae*** (=*Nosema corneae*), ***Tubulinosema acridophagus***, and an unknown species likely belonging to ***Endoreticulatus***.

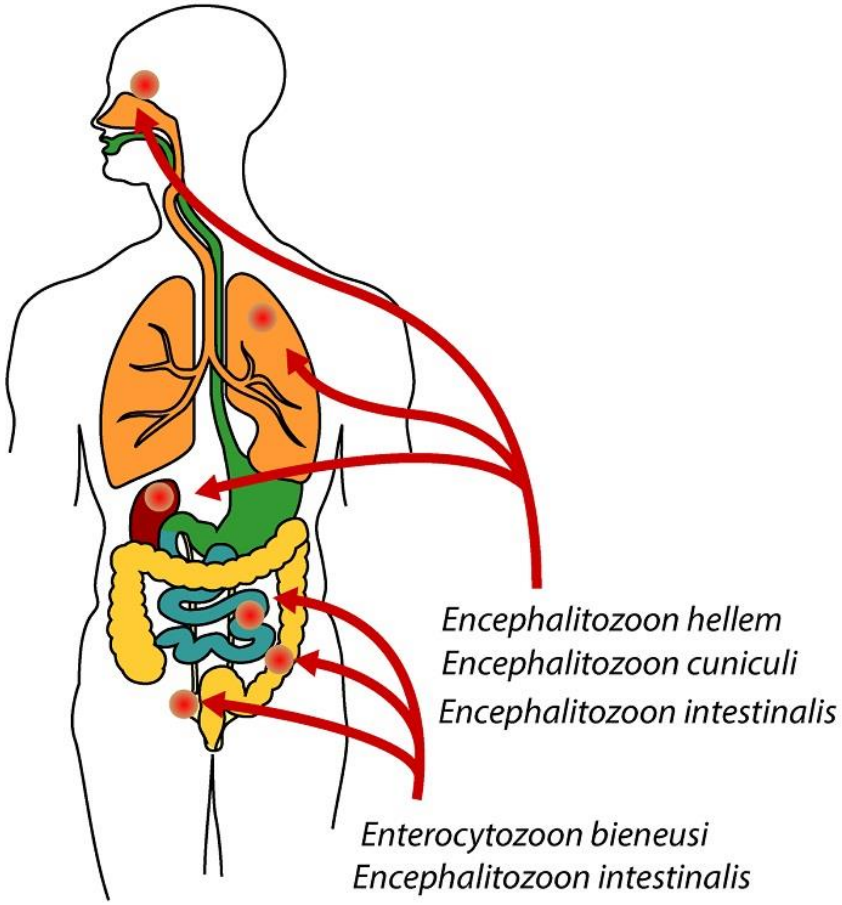
Life Cycle

- The infective form of microsporidia is the resistant spore, which can persist in the environment for months image The spore then germinates, rapidly everting its polar tubule which contacts the eukaryotic host cell membrane image . The spore then injects the infective sporoplasm into the host cell through the polar tubule image . Inside the cell, the sporoplasm enters the proliferative phase marked by extensive multiplication via merogony (binary fission or multiple fission), creating meronts image . The location of this developmental stage within the host cell varies by genus; it can occur either in direct contact with the host cell cytosol (Enterocytozoon, Nosema), inside a parasitophorous vacuole of unknown origin (Encephalitozoon), in a parasite-secreted envelope (Pleistophora, Trachipleistophora), or surrounded by the host cell endoplasmic reticulum (Endoreticulatus, Vittaforma) image . Following the proliferative phase, meronts undergo sporogony in which the thick spore wall and invasion apparatus develop, creating sporonts and eventually mature spores when all organelles are polarized. When the spores increase in number and completely fill the host cell cytoplasm, the cell membrane is disrupted and spores are released to the surroundings image . These free mature spores can infect new cells thus continuing the cycle.
- Mature spores of intestinal-localizing species may be shed in feces, although the route of transmission remains uncertain for many species. Exposure to spores in water or in soil appears to be a potentially major route, based on the finding of spores in these sources along with case histories. *E. bieneusi* and *V. corneae* have been identified in surface waters, and spores of *Nosema* sp. (and likely *A. algerae*) have been identified in ditch water. Cases of donor-derived microsporidiosis (*Encephalitozoon cuniculi*) following bone marrow, kidney, liver, and heart transplantation have been confirmed.

Microsporidia

Intracellular development

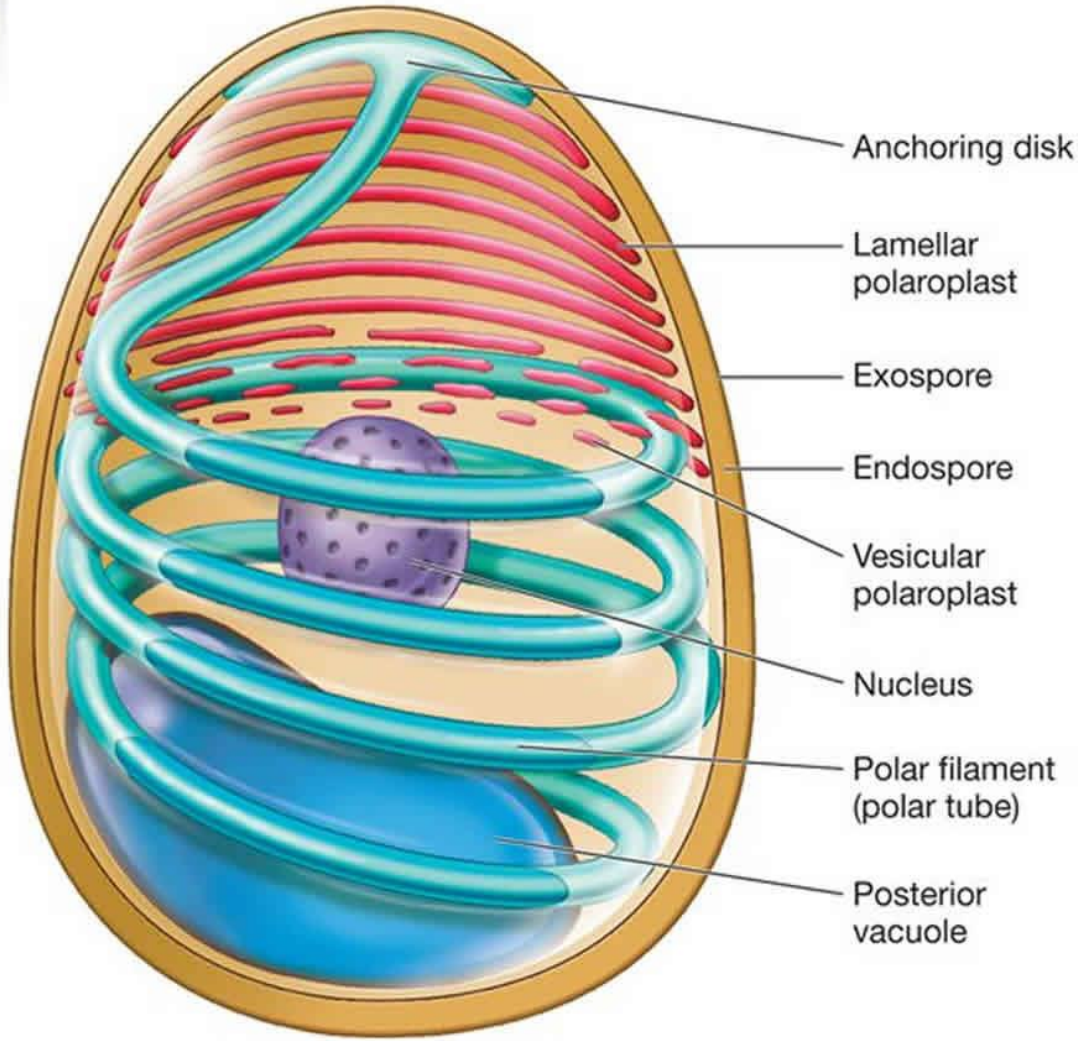




Clinical Presentation

- Human microsporidiosis represents an important and rapidly emerging opportunistic disease. Historically it has been observed in severely immunocompromised persons, particularly among persons with AIDS, however the implementation of effective anti-retroviral therapies has reduced the incidence in this group considerably. Cases are also known to occur in immunocompetent individuals. The clinical manifestations of microsporidiosis are very diverse, varying according to the causal species and route of infection. Disseminated infection can be fatal. Of all of the manifestations of microsporidiosis, *Enterocytozoon bieneusi*-associated diarrhea is the most common. Below is a table summarizing the typical sites of infection for various species:

Microsporidia Pathogenesis



- Human infections
  - *Enterocystozoon bieneusi*
    - diarrhea
    - pneumonia
  - *Encephalitozoon cuniculi*
    - encephalitis
    - nephritis
  - severe in HIV/AIDS patients

Laboratory diagnosis

•Microscopy

•Light microscopic examination of the stained clinical smears, especially the fecal samples, is an inexpensive method of diagnosing microsporidial infections even though it does not allow identification of microsporidia to the species level. The most widely used staining technique is the Chromotrope 2R method or its modifications. This technique stains the spore and the spore wall a bright pinkish red. Often, a darker-staining equatorial band is seen in the middle of the spore. This technique, however, is lengthy and time consuming and requires about 90 min. A recently developed "Quick-Hot Gram Chromotrope technique" however, cuts down the staining time to less than 10 min and provides a good differentiation from the lightly stained background fecal materials so that the spores stand out for easy visualization. The spores stain dark violet and the equatorial band is enhanced. In some cases dark staining Gram positive granules are also clearly seen. Chemofluorescent agents such as Calcofluor white are also useful in the quick identification of spores in fecal smears. The spores measure from 0.8 to 1.4 µm in the case of *Enterocytozoon bieneusi*, and 1.5 to 4 µm in *Anncaliia algerae*, *Encephalitozoon* spp., *Vittaforma corneae*, and *Nosema* spp.

•Transmission electron microscopy (TEM) is still the gold standard and is necessary for the identification of the microsporidian species, which is based on internal features of the spore such as the number of polar tubule coils. However, TEM is expensive, time consuming, and not feasible for routine diagnosis.

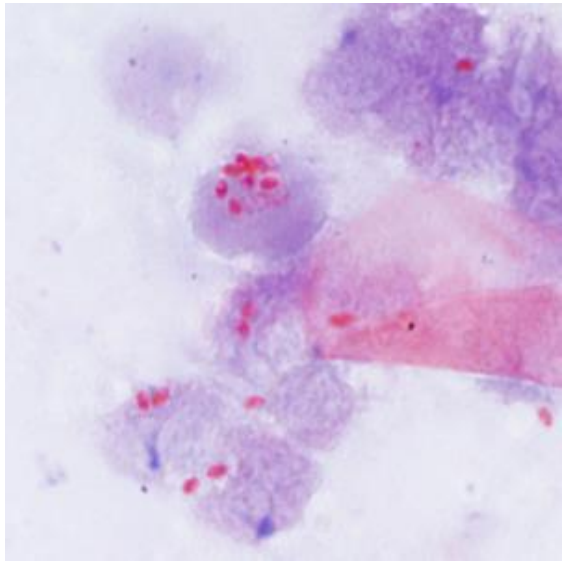
•Immunofluorescence Assays (IFA)

•IFAs are available for microsporidia using monoclonal and/or polyclonal antibodies.

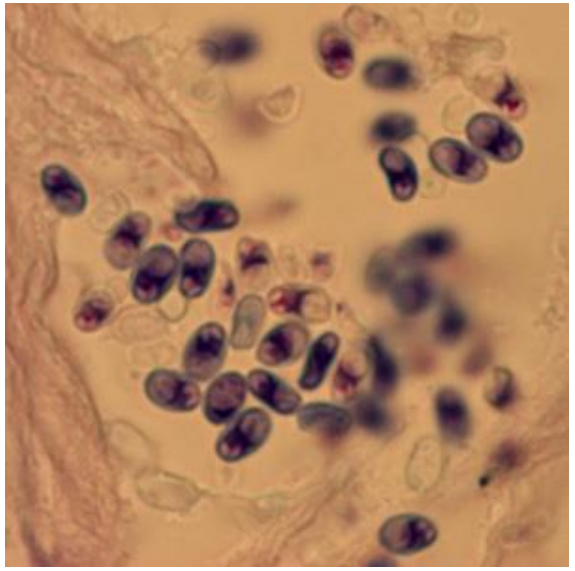
•Molecular Methods (PCR)

•The CDC offers molecular identification of *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon hellem* and *Encephalitozoon cuniculi* using species-specific polymerase chain reaction (PCR) assays. Molecular identification of other microsporidia species can be attempted using genera-specific primers and sequencing analysis on a case-by-case basis.

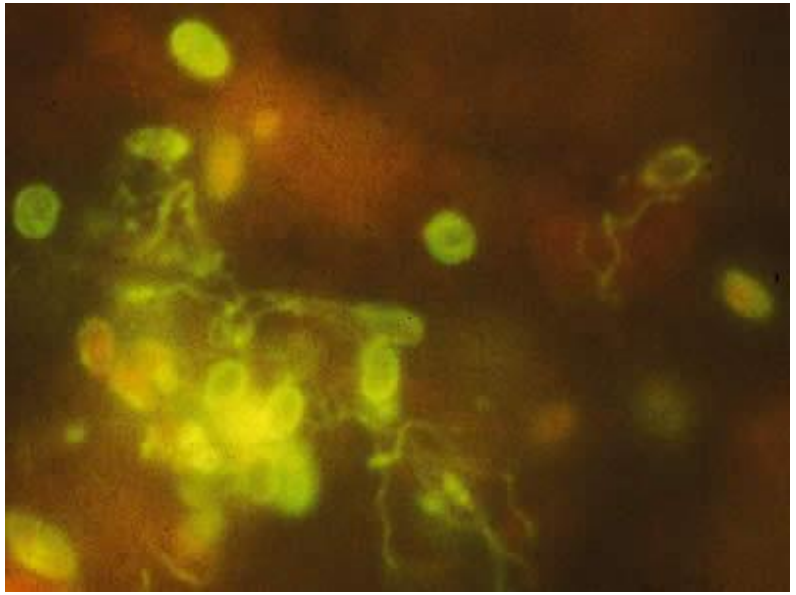
Laboratory diagnosis



Trichrome blue



Giemsa



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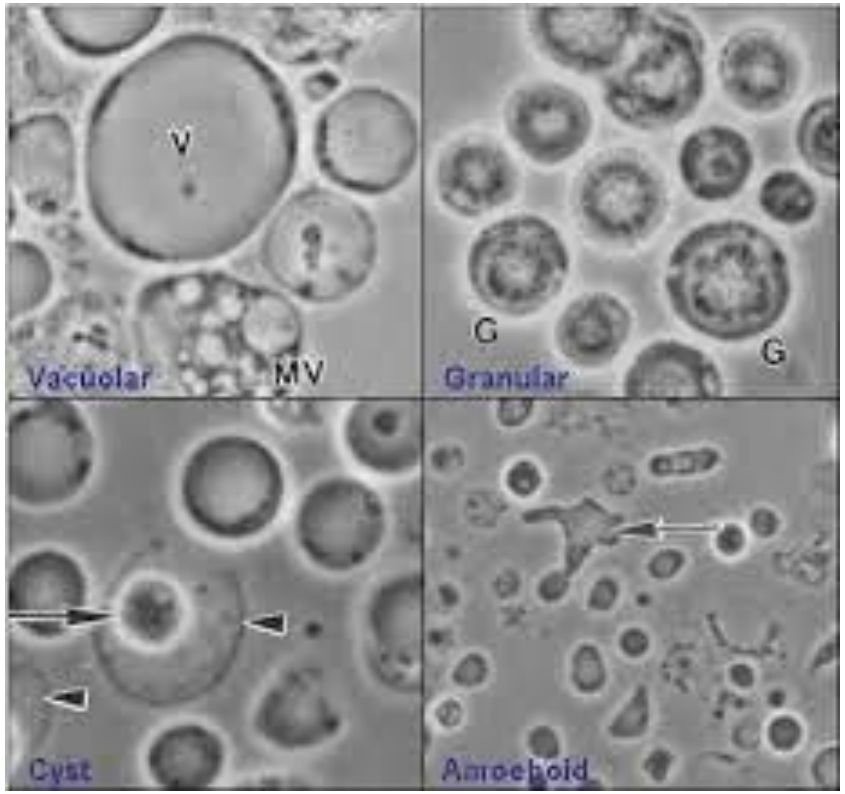
Blastocystis sp.

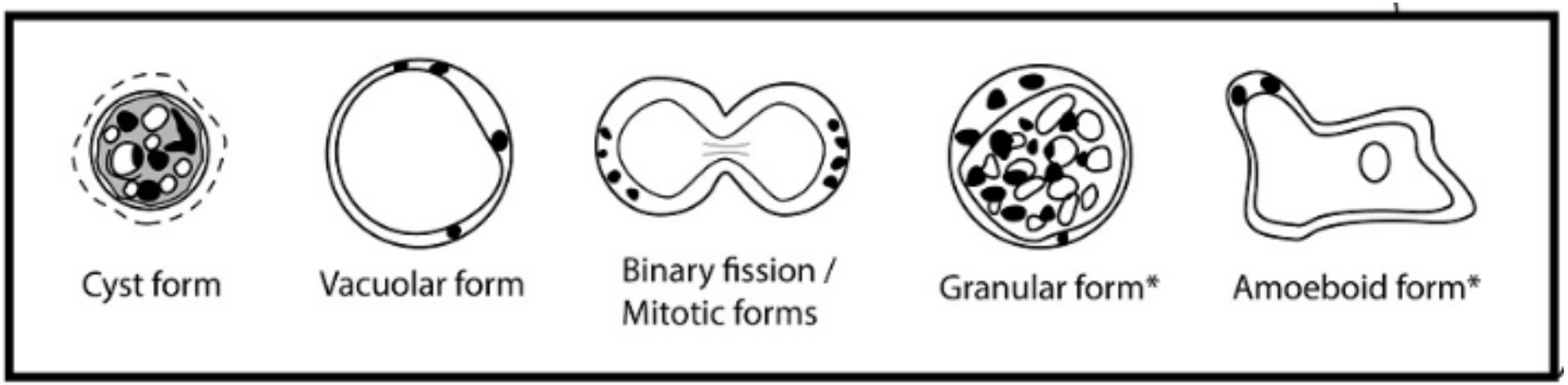
The genus Blastocystis belongs to the order Blastocystida of the class Blastocystea of the phylum Bygira. Previously, blastocysts were thought to be yeast fungi. Currently, they are considered protozoa.

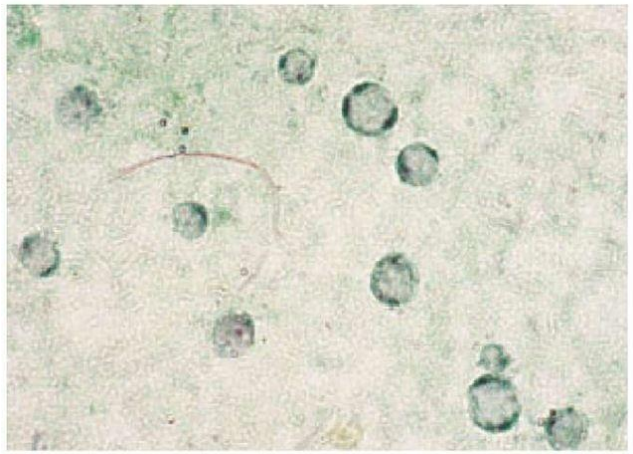


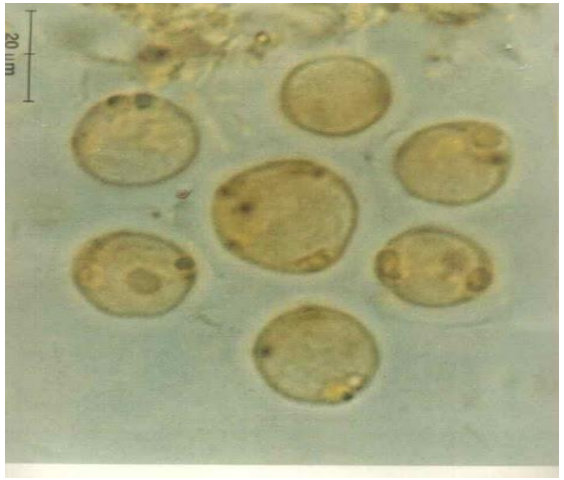
- (Kingdom): Chromista
- **(Phylum):**Bigyra
- (Class): **Blastocystea**
- (Order): **Blastocystida**
- (Family): **Blastocystidae**
- (Genus): ***Blastocystis***
- ***(Species): Blastocystis*** hominis

Blastocystis sp.

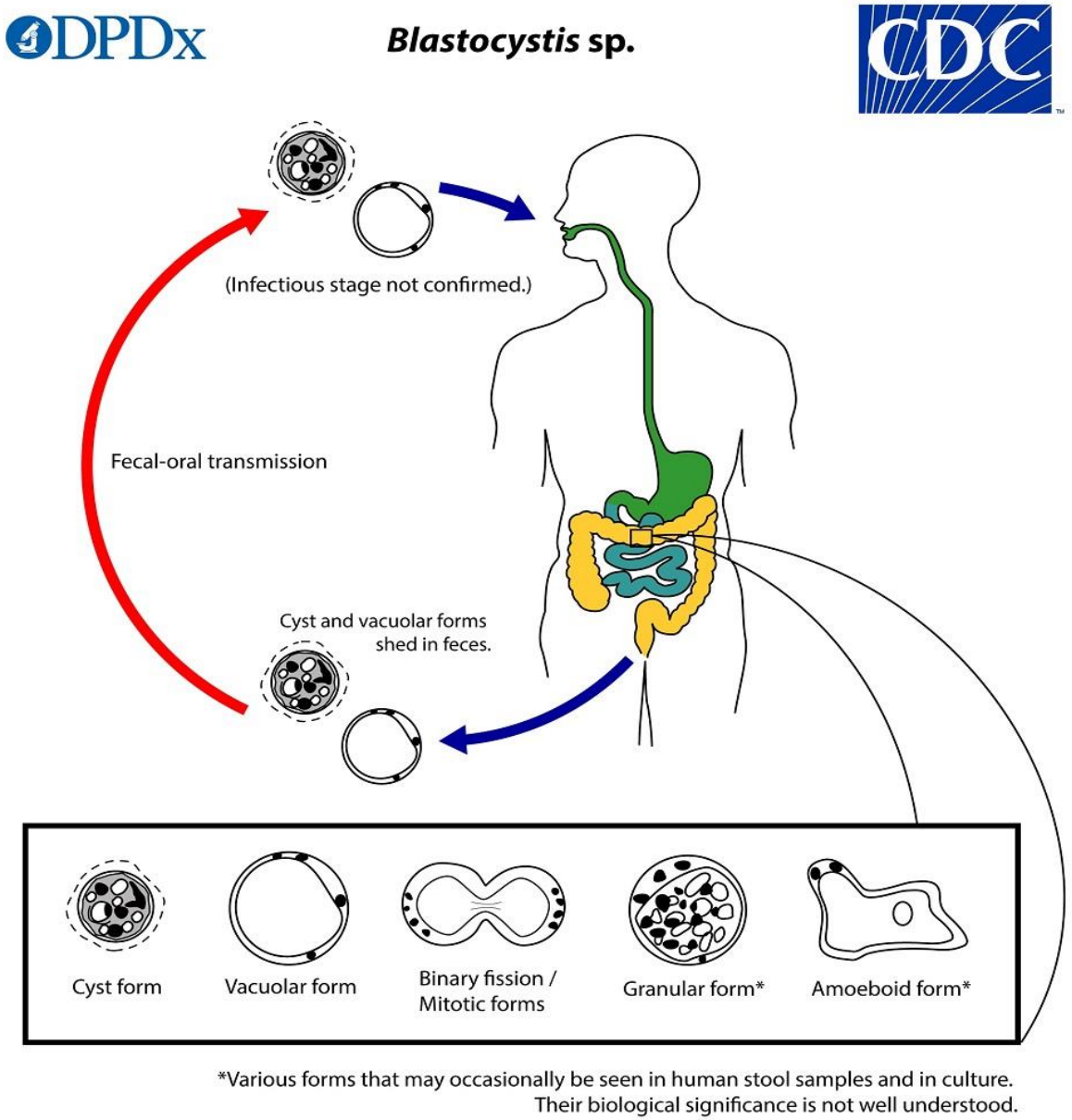
- Blastocysts are 5-30 µm spherical, polymorphic primitives, similar to amoebae, and can form pseudopodia. The nucleus of the parasite is pushed to the periphery by a vacuole-like body in the cytoplasm. It feeds on bacteria and reproduces by dividing into two.







Source of infection, mode of transmission, pathogenesis, clinic and diagnosis



The **source** of infection is humans and animals. Cyst and vacuolar forms of the parasite enter the body with **food** and **water**.

They often cause ***blastocystosis***, which is accompanied by asymptomatic diarrhea and sometimes diarrhea.

Diagnosis is based on microscopy of stool smears. The presence of 5 or more parasites in the field of vision in preparations made from faeces ("crushed drop") is considered a diagnostic sign.

TRICHOMONAS

CLASSIFICATION

- **Domain : Eukarya**
- **Kingdom` : Protista**
- **Phylum : Metamonada**
- **Class : Parabasilia**
- **Family : Trichomonadida**
- **Genus : Trichomonas**
- **Species : Trichomonas vaginalis**

GENERAL PROPERTIES

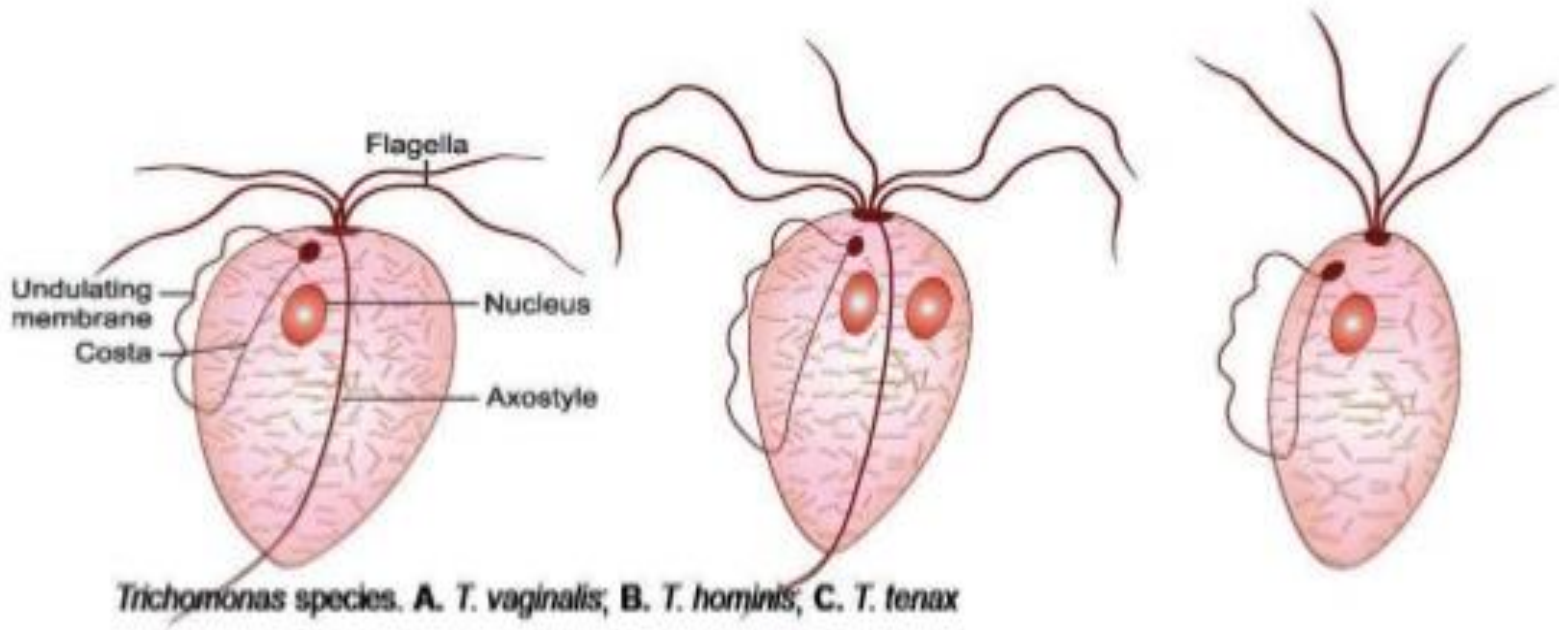
- Characteristically have a cytostome,
- An anterior tuft of flagella,
- Undulating membrane with the recurrent flagellum,
- An axostyle protruding through the posterior end ,
- Only trophozoite stage.

Genus *Trichomonas* has 3 species, which occur in humans

T. vaginalis

T. hominis

T. tenax



Trichomonas species. **A.** *T. vaginalis*; **B.** *T. hominis*; **C.** *T. tenax*

1. Trichomonas vaginalis : found in vagina, urethra and prostrate, infection is pathogenic.

2. Trichomonas tenax : found in oral cavity occurring particularly in dental cavities and at the gingival margins, infection is non pathogenic.

3. Pentatrichomonas hominis : found in lower GI tract particularly in caecum, non pathogenic.

Trichomonas vaginalis

- Important species which causes infection in both male and females.
- Is the causative agent of trichomoniasis, infection is sexually transmitted.
- In 1837 Donne first observed the flagellate.

MORPHOLOGY

- Exists only in trophozoite stage, cystic stage is absent.

- **TROPHOZOITE**
- Is pear shaped

- Measures 7 to 23 micrometer in length.

- Twitching motility.

- Four anterior free flagella, arising from a shallow depression in the anterior end of the body called periflagellar canal.

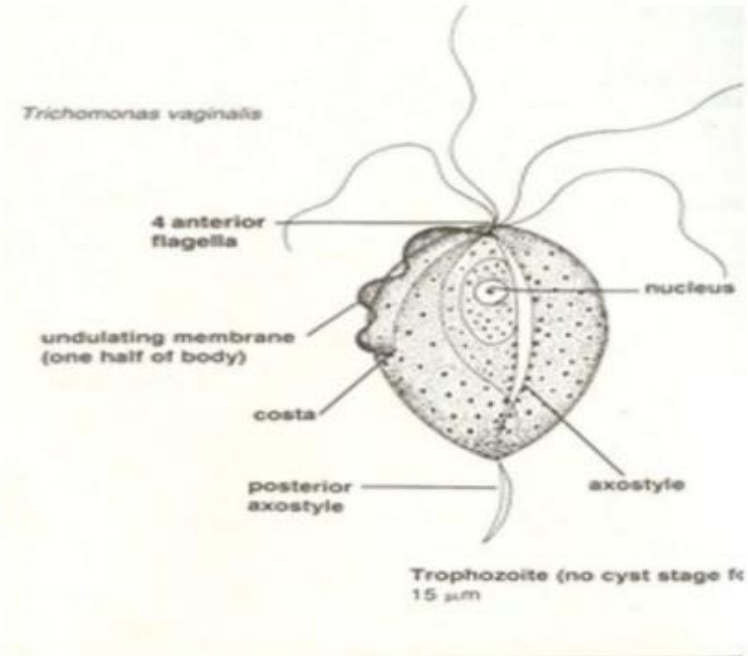
- Fifth flagellum curve back along the margin of the undulating membrane and is called the recurrent flagellum.

- Costa is the rigid cord, filamentous and support to the undulating membrane.

- An axostyle is a hyaline rod like structure that runs through the centre of the body and comes out at the posterior end.

- An axostyle is a part of the endoskeleton.

- The cytoplasm contains a large numbers of siderophilic granules and sometimes viral particles.



MODE OF TRANSMISSION

- Trophozoite cannot survive outeside and so infection has to be transmitted directly from person to person.
- Sexual transmission is the usual mode of transmission.
- Trichomoniasis often coexists with other sexually transmitted diseases; like candidiasis, gonorrhea, syphillis, or human immunodeficiency virus (HIV).

CLINICAL MANIFESTATION

- STI
- Common cause of vaginitis in women and urethritis in Men.

- Trichomoniasis presents a wide variety of clinical patterns. The spectrum of clinical trichomoniasis in women ranges from the asymptomatic carrier state to flagrant vaginitis, with 1/3rd of the asymptomatic infected patients becoming symptomatic within 6months.

WOMEN (SYMPTOMATIC)

- Vulvo vaginitis (Trichomonal vaginitis)
- Urethritis

IN MEN (ASYMPTOMATIC)

- Urethritis, epididymis, prostatitis, and superficial penile ulcerations.
- Irritation inside the penis, mild discharge, discharge may be purulent to mucoid or slight burning after urination or ejaculation.
- Mostly self limiting trichomonal action of the prostatic fluid or flushing out of the flagellate during micturation.

EPIDEMIOLOGY

- Most common non viral sexual transmitted disease.
- An estimated 200 million women suffer from trichomoniasis every year worldwide.
- Prevalence of trichomoniasis varies between 5% in patients at hospital to 75% in sexual workers.

SPECIMENS

- **IN WOMEN :** vaginal discharge, endocervical specimens.

- **IN MEN:** Prostatic fluid, less commonly semen.

- **common specimens** urethral swab, early morning first voided urine sediment.

LAB DIAGNOSIS

1. MICROSCOPY
2. CULTURE
3. ANTIGEN DETECTION IN VAGINAL SMEARS
4. MOLECULAR DIAGNOSIS
5. OTHER TESTS

1. MICROSCOPY

- Trichomonas in the vaginal discharge can be demonstrated by;
    - Wet mount
    - Acridine orange staining
    - Papanicolau stain (PAP smear)
    - Direct fluorescent antibody (DFA)staining

- Vaginal or urethtral discharge is examined microscopically in saline wet mount preparation for characteristic, jerky and twitching motility and shape. In males trophozoites may be found in urine or prostatic secretions.

- Fixed smears may be stained with acridine orange , Papanicolaou stain.

- DFA is more sensitive.

2. CULTURE

- Consider as gold standard for the diagnosis.
- Is recommended when direct microscopy is negative and is considered as **gold standard** as well as the most sensitive (95%) method for the diagnosis of Trichomonas vaginalis infection.
- Grows best at 35-37degree celsius under anaerobic condition.

- The optimal pH for growth is 5.5- 6.

- Can be grown in a variety of solid or liquid media , tissue culture, and eggs. Cystein-peptone-liver-maltose(CPLM) medium and plastic envelope medium (PEM) are often used.

3. ANTIGEN DETECTION IN VAGINAL SMEARS

- ELISA is used for demonstration of Trichomonas antigen in vaginal specimens.

- ELISA using a monoclonal antibody specific for a 65k Da surface polypeptide of Trichomonas vaginalis is a very specific and sensitive method for detection of parsaite in vaginal secretion.

4. MOLECULAR DIAGNOSIS

- DNA probes

  synthetic oligo nucleotide probes

- PCR

  highly sensitive (97%) and specific (98%) test for the diagnosis of Trichomonas vaginalis.

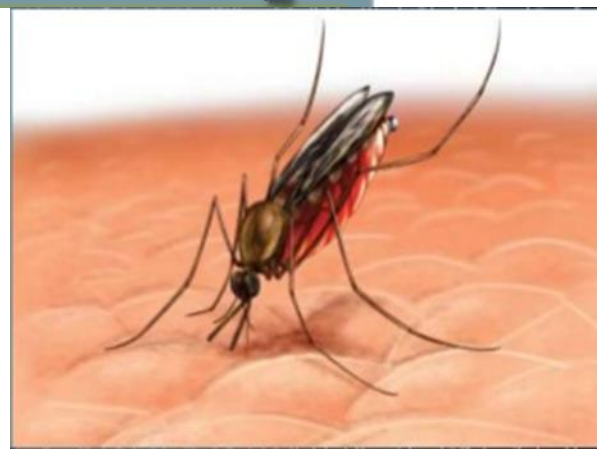
TREATMENT

- Simultaneous treatment of both patners is recommended.
- Metronidazole 2g orally as a sigle dose or 250nmg three times daily for 7 days.
- Metronidazole is contraindicate in pregnancy due to is mutagenecity, so topical therapy with clotrimazole is applied.

PREVENTION AND CONTROL

- Safe sexual behaviour.
- Avoidance of multiple sex patners.
- Use of condom.
- Detection and treatment of cases either male or female.

Malaria parasite



Introduction

- Malaria is the mosquito born infectious disease of human. Malaria is caused by a parasite that is passed from one human to another by the bite of infected *Anopheles* mosquitoes. After infection, the parasites (called sporozoites) travel through the bloodstream to the liver, where they mature and release another form, the merozoites. which introduces the protists via its saliva into the circulatory system, and ultimately to the liver where they mature and reproduce. The disease causes symptoms that typically include fever and headache, which in severe cases can progress to coma or death.

- **Malaria parasite**

The Parasite which cause malaria in man and other animals belong to -

Class:- Sporozoa

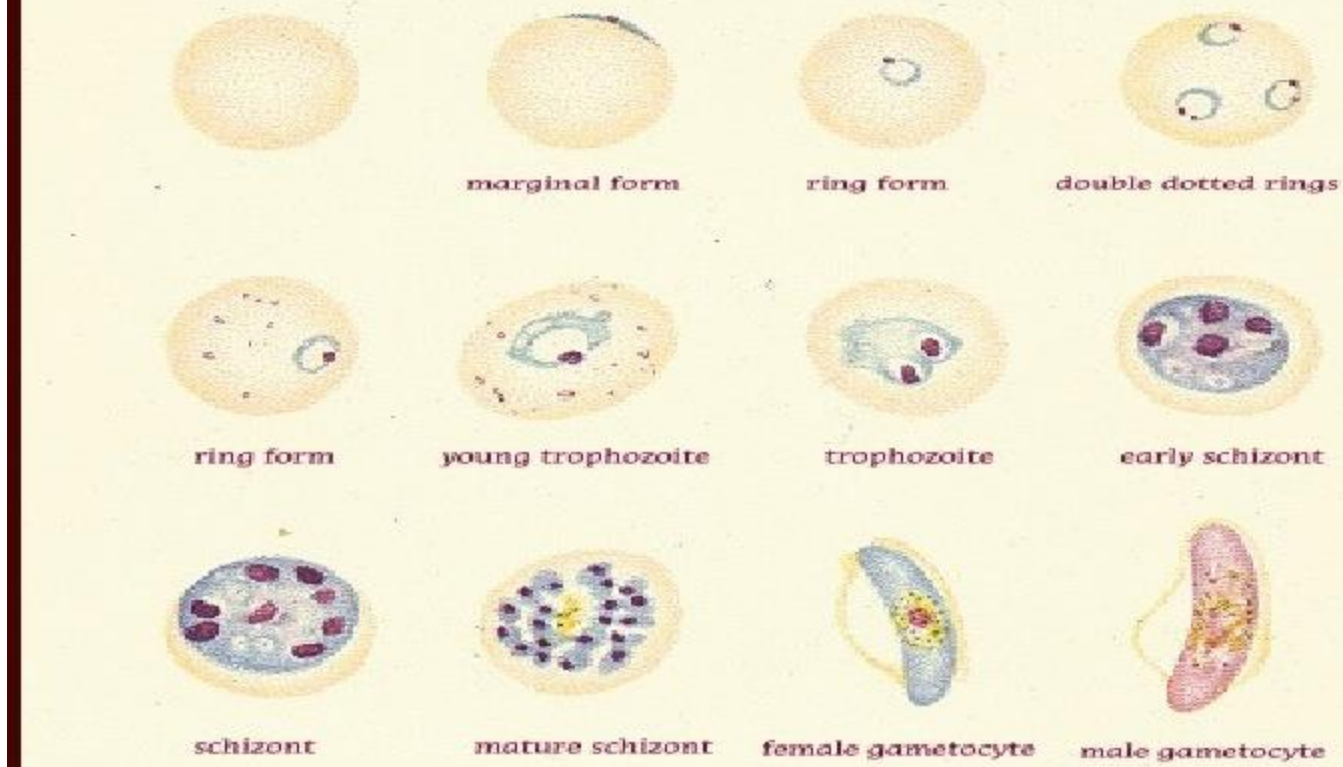
Suborder:- Hemosporidia

Genus:- Plasmodium

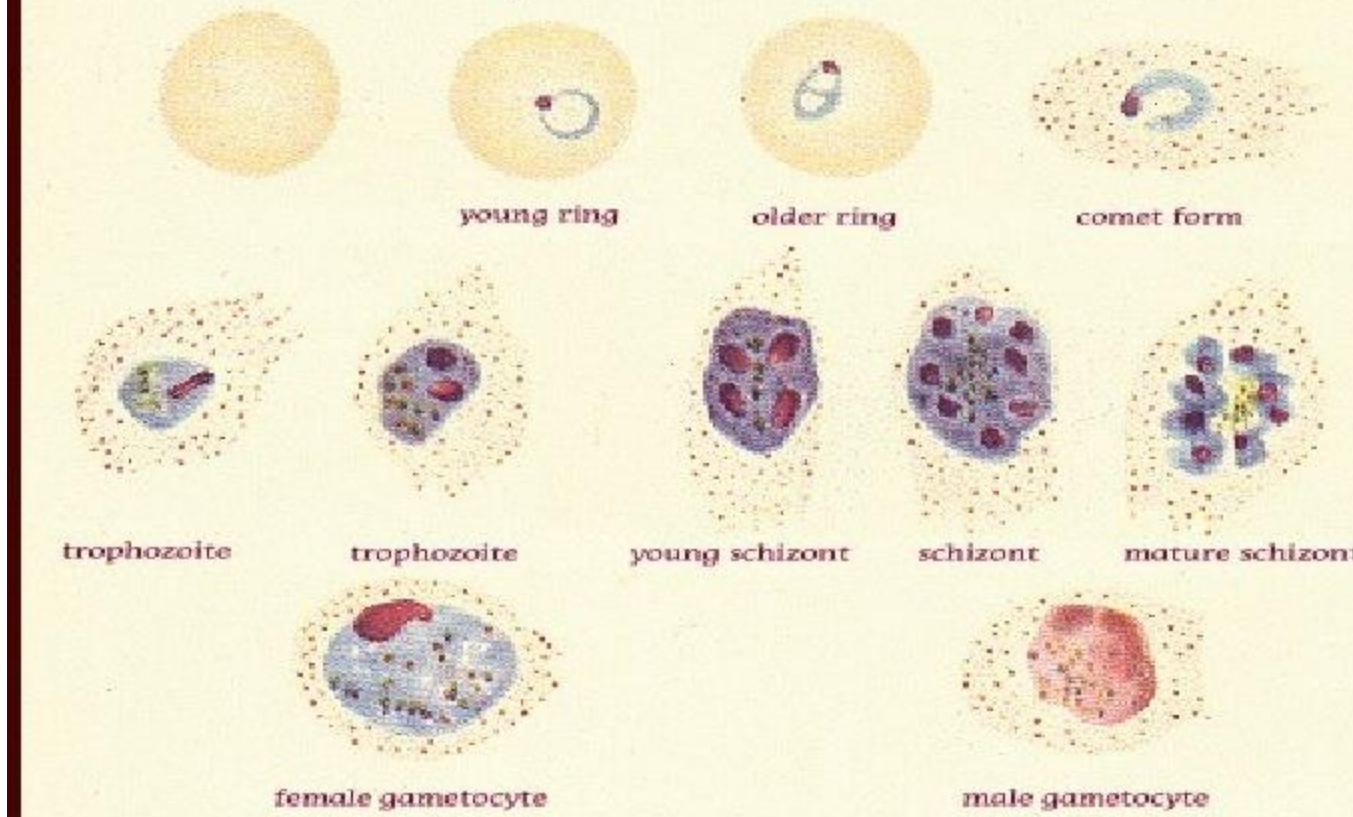
Most common species found in man are :-

1. Plasmodium Vivax
2. Plasmodium Falciparum
3. Plasmodium Malariae
4. Plasmodium ovale

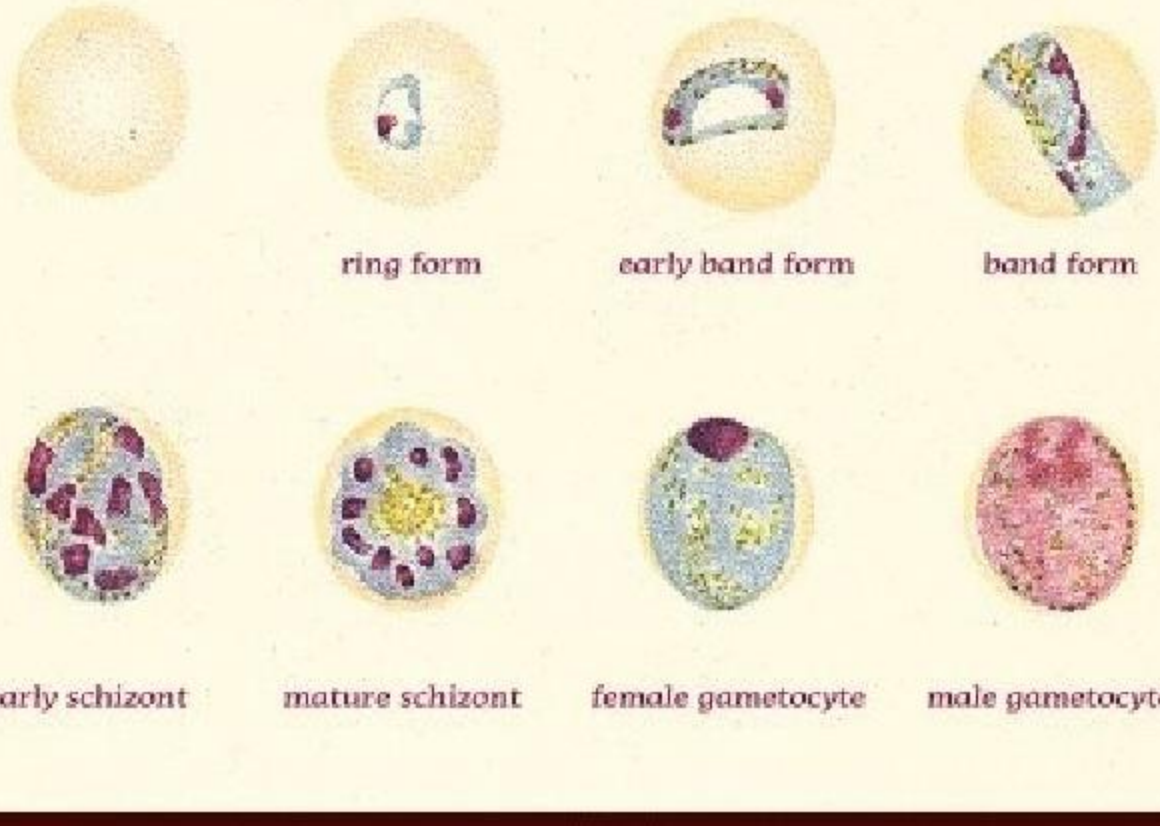
P. falciparum



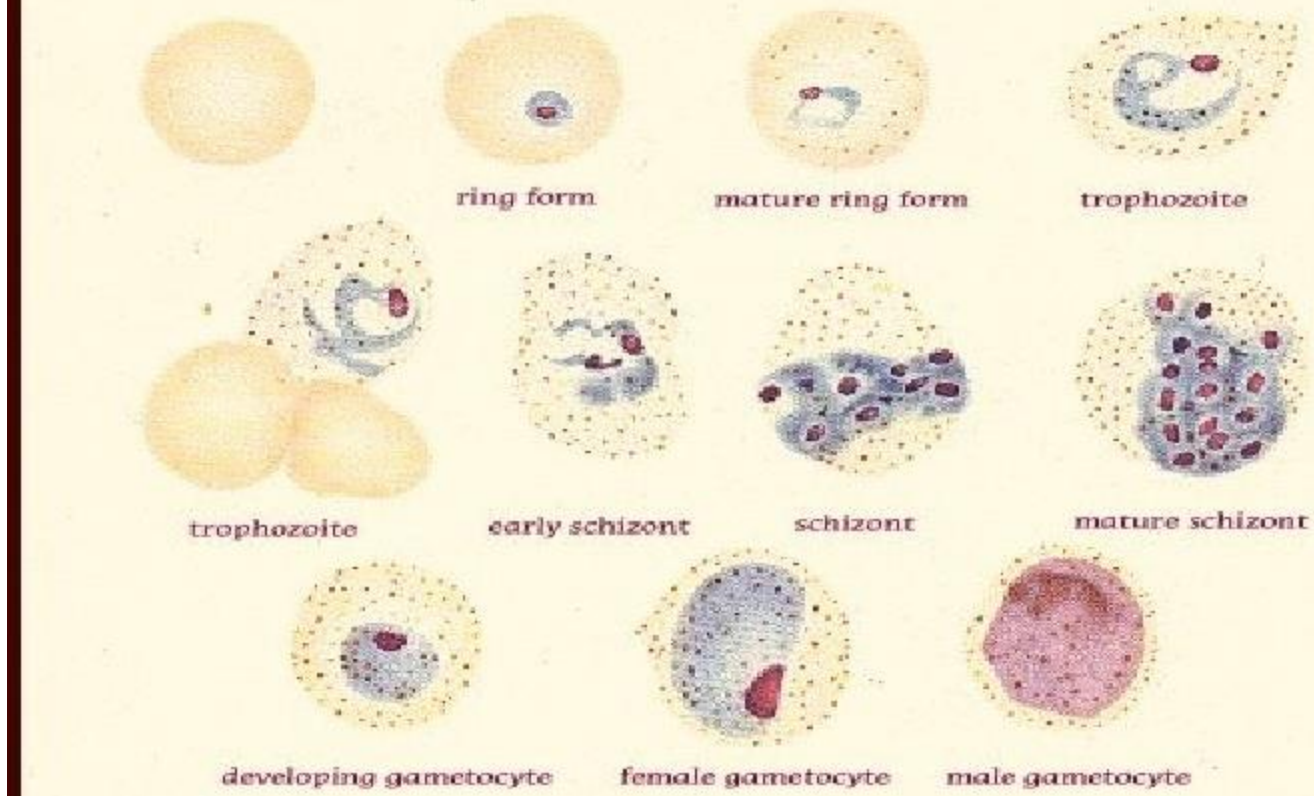
P. ovale


young ring
older ring
comet form
trophozoite
trophozoite
young schizont
schizont
mature schizont
female gametocyte
male gametocyte

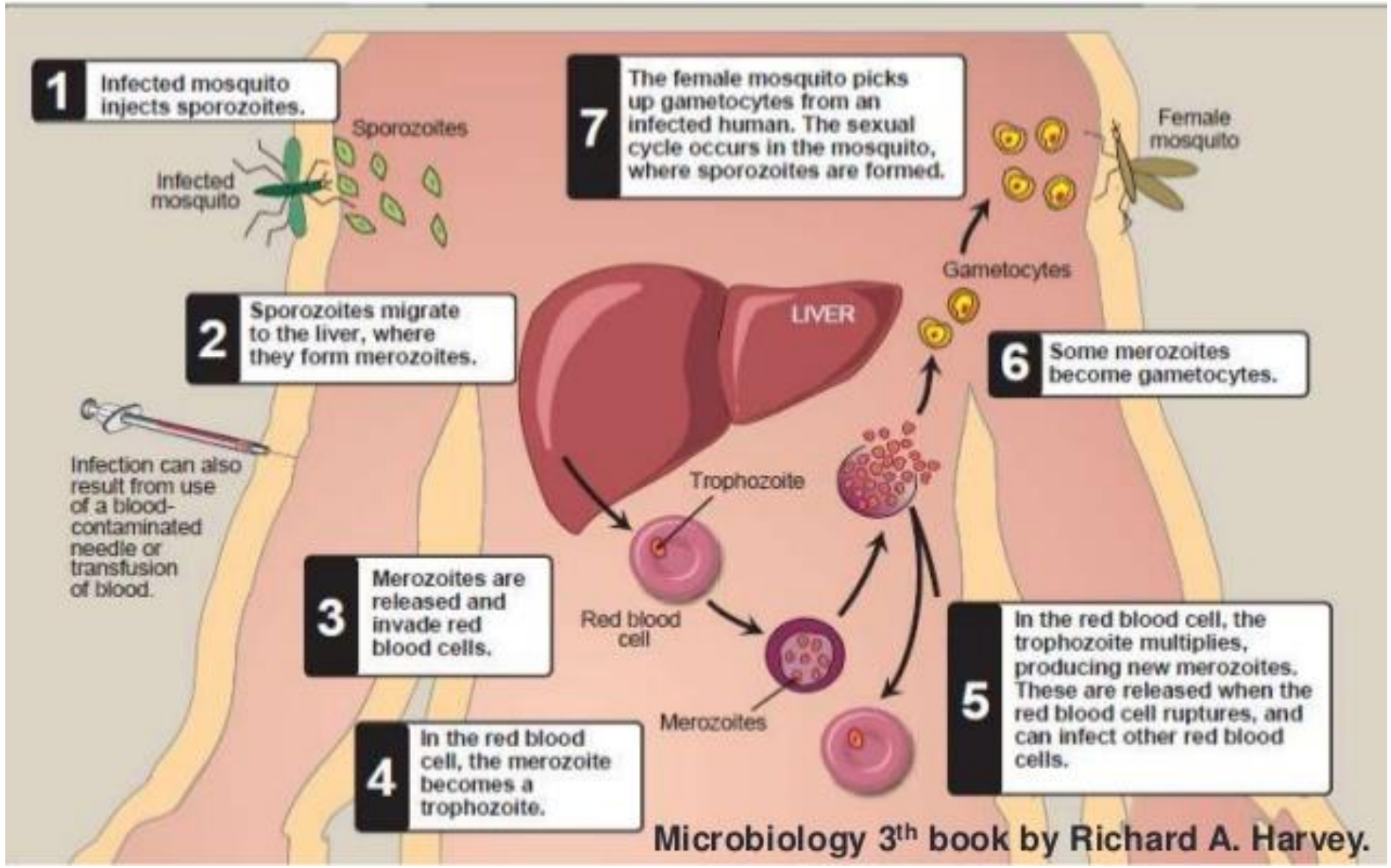
P. malariae


ring form
early band form
band form
early schizont
mature schizont
female gametocyte
male gametocyte

P. vivax


ring form
mature ring form
trophozoite
trophozoite
early schizont
schizont
mature schizont
developing gametocyte
female gametocyte
male gametocyte

Life cycle of malaria



Incubation period of the parasite

species	Incubation period (Liver cycle)
P. falciparum	7-14 days
P. vivax	12-17 days (with relapse up to 3 years)
P. ovale	9-18 days (with relapse up to 20 years)
P. malaria	13-40 days.

Con…

- The time between the fever episodes can be characteristics of the infecting plasmodium species.

species	Duration of fever (erythrocytic cycle)
P. falciparum	36-48 h, Malignant tertian malaria.
P. vivax	48h, Benign tertian malaria.
P. ovale	48h, Ovale tertian malaria.
P. malaria	72h, Quartan malaria.

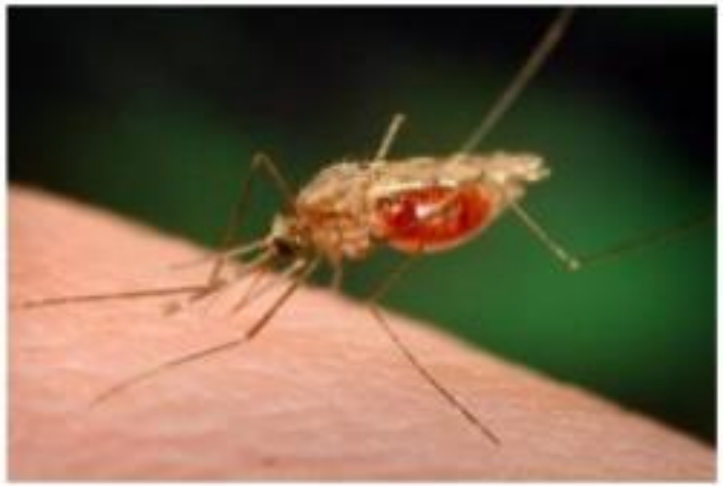
Pathophysiology of malaria

- Showers of new merozoites are released from the RBCs at intervals of approximately 48h for P.vivax, P.ovale and P.falciparum and 72h for P.malaria. The episodic shaking, chills, and fever coincide with this release.

- The parasites destroy large numbers of infected RBC, thereby causing a hemolytic anemia.

How is malaria transmitted?

- Malaria parasites are transmitted from one person to another by the bite of a female anopheles mosquito.
- The female mosquito bites during dusk and dawn and needs a blood meal to feed her eggs.
- Male mosquitoes do not transmit malaria as they feed on plant juices and not blood.
- There are about 60 species of anopheles are able to transmit malaria.
- Like all mosquitoes, anopheles breed in water - hence accumulation of water favours the spread of the disease.

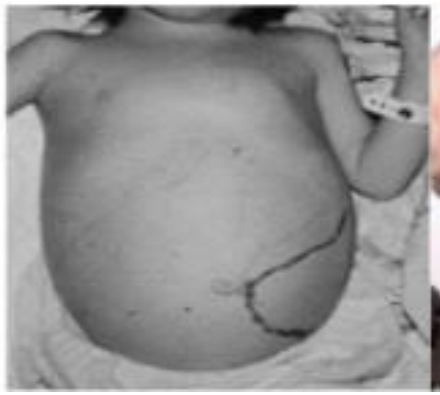


Symptoms of malaria

Fever **Sweating** **Chills**

Signs and symptoms of malaria

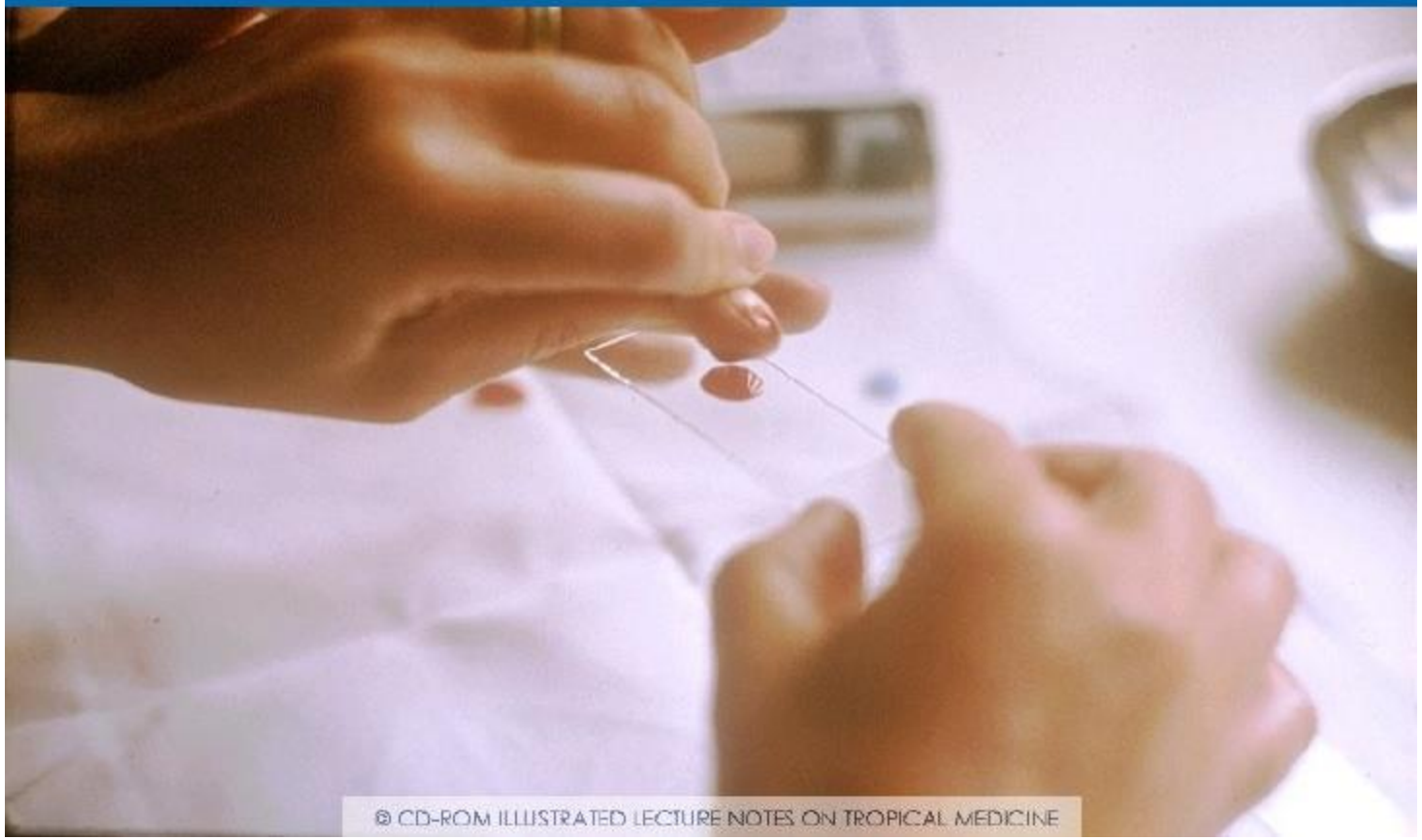
- Cycles of shaking chills followed by fever and profuse sweating.
- Hemolytic anemia.
- Jaundice.
- Dark pigmented urine (blackwater fever)
- Stools became increasingly loose.
- Hepatomegaly.
- Splenomegaly.
- Headache.
- Dry cough.
- Lost weight.



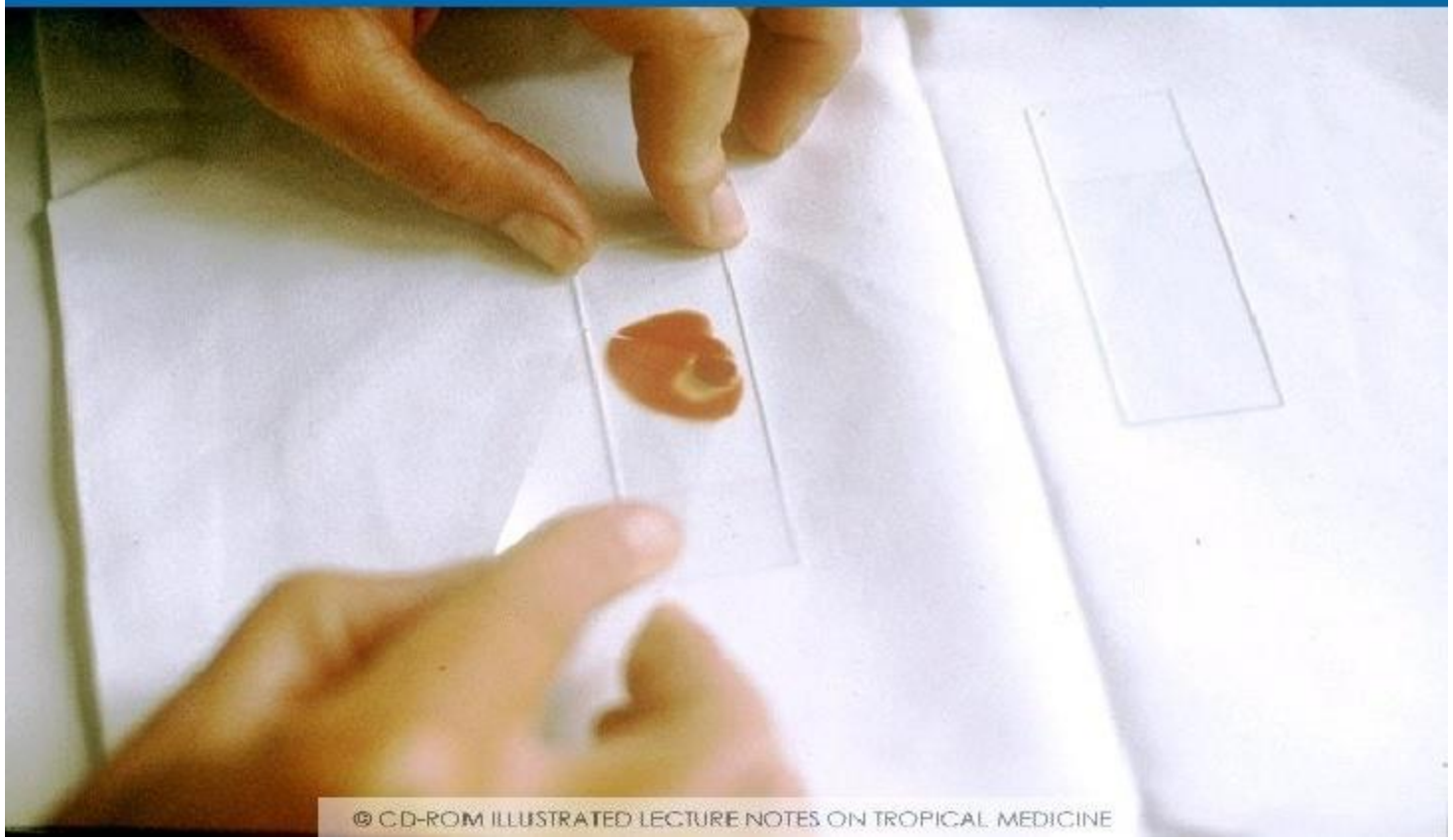
Laboratory Diagnosis

- Laboratory diagnosis of malaria can be made through microscopic examination of thick and thin blood smears. Thick blood smears are more sensitive in detecting malaria parasites because the blood is more concentrated allowing for a greater volume of blood to be examined; however, thick smears are more difficult to read

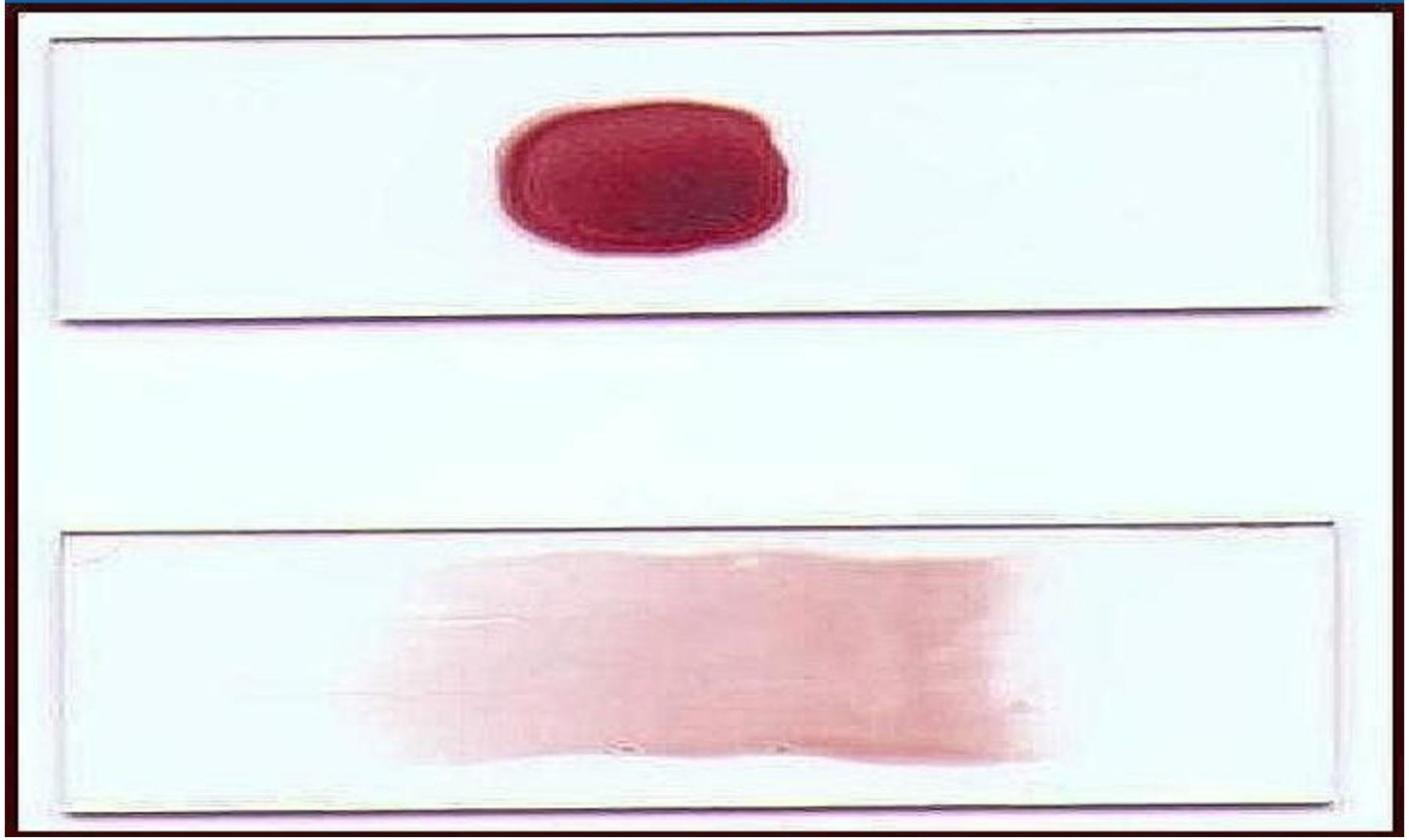
Making of Thick smear



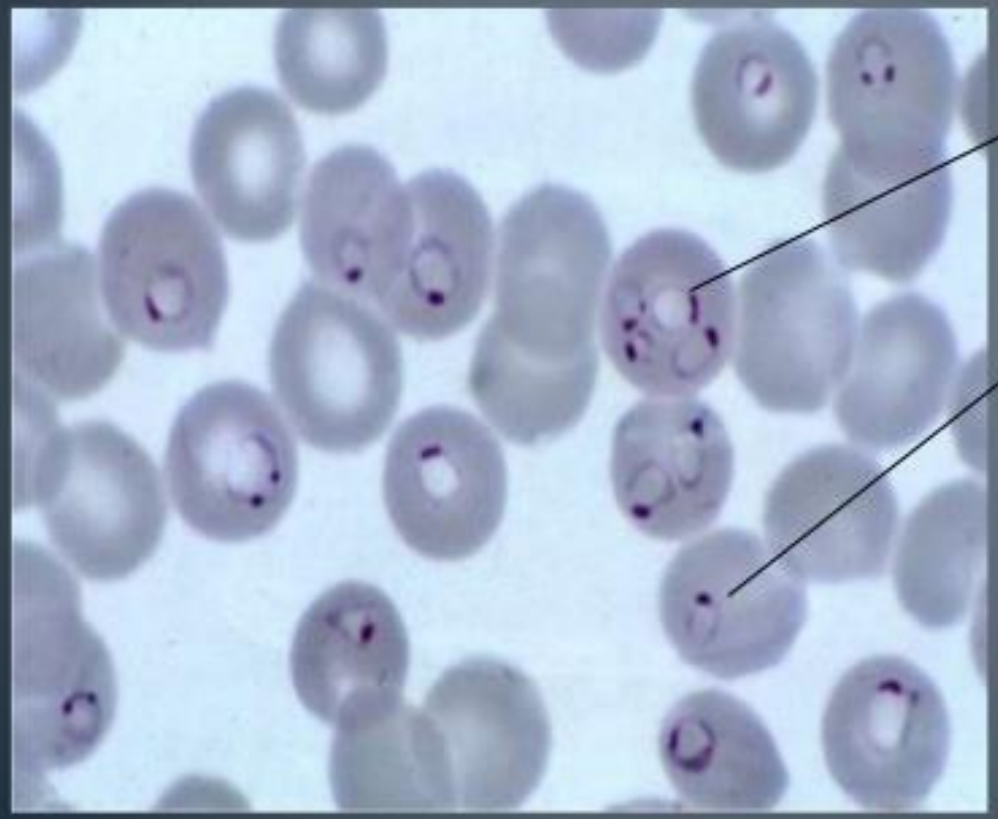
How a thick smear looks



Appearance of Thick and Thin Smears



Appearance in blood film in Microscope



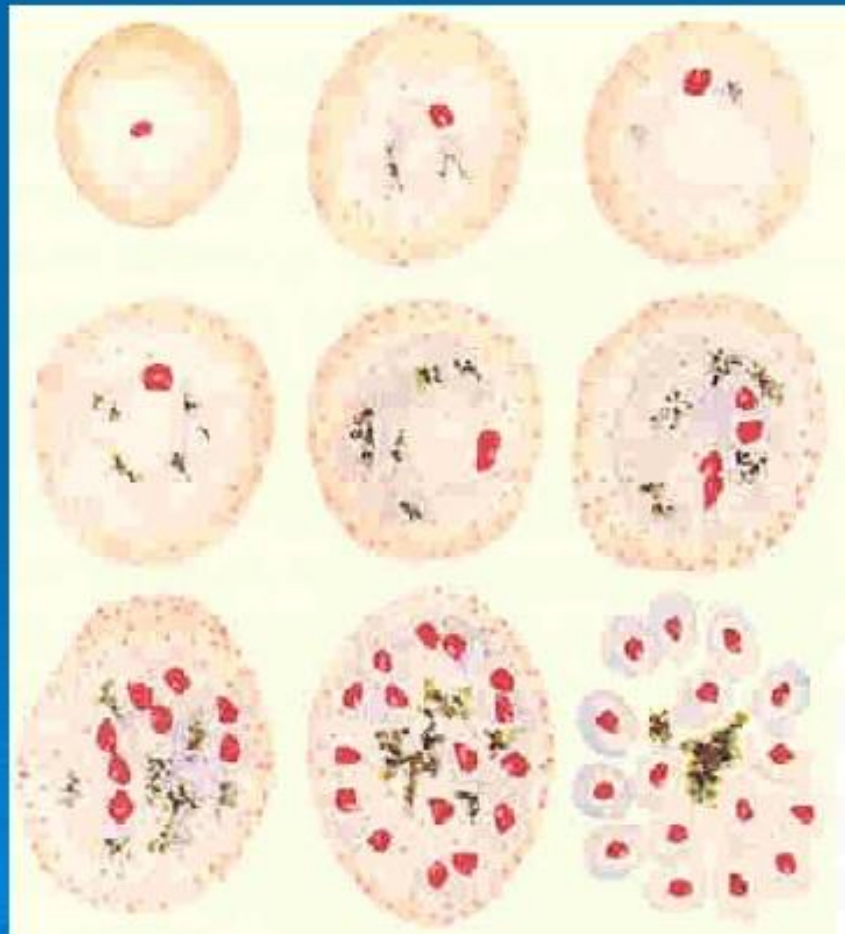
Ring forms or trophozoites; many

red cells infected –

some with more than one parasite

Microscopic demonstration still the Gold standard in Diagnosis

Blood smear stained with Giemsa's stain



Antigen Detection Methods are Rapid and Precise

- **Antigen Detection**
- Various test kits are available to detect antigens derived from malaria parasites. Such immunologic ("immunochromatographic") tests most often use a dipstick or cassette format, and provide results in 2-15 minutes. These "Rapid Diagnostic Tests" (RDTs) offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not available. Malaria RDTs are currently used in some clinical settings

Result of Rapid Malaria test

Malaria P.f. RDT Results

NEGATIVE RESULTS

Wait 15 minutes before reading results

POSITIVE RESULTS

INVALID RESULTS *

* No Control Lines (repeat tests)

Serology

- Serology detects antibodies against malaria parasites, using either indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA). Serology does not detect current infection but rather measures past experience.

Newer Diagnostic methods

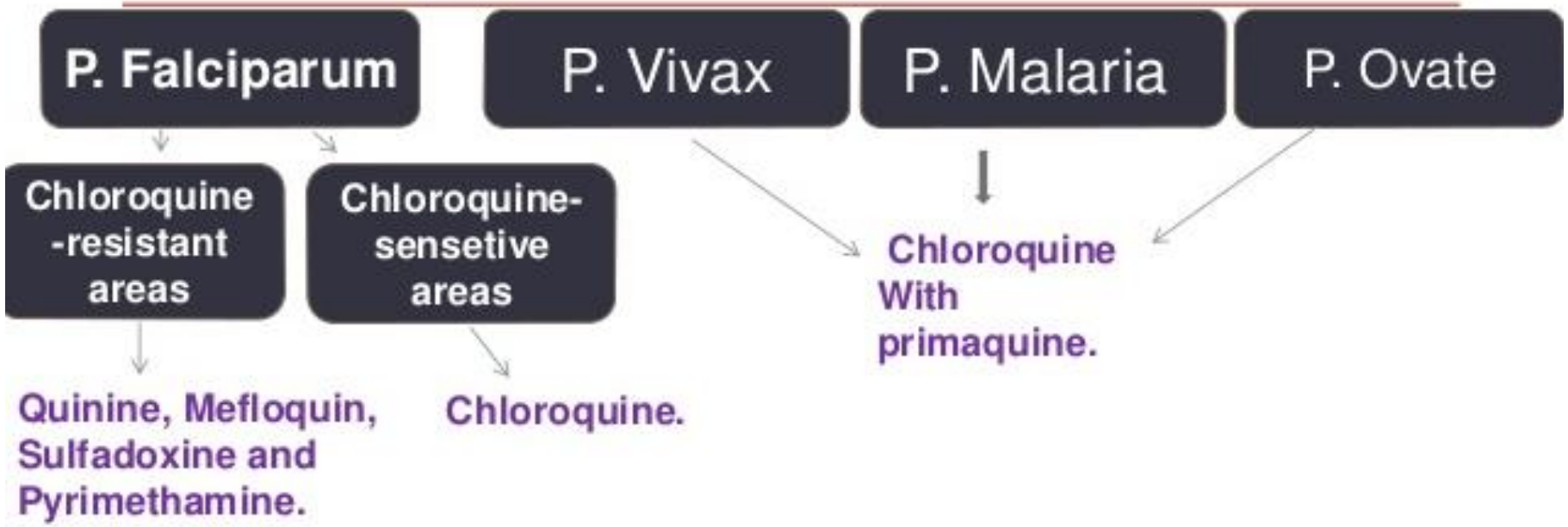
Molecular Diagnosis

- Parasite nucleic acids are detected using polymerase chain reaction (PCR). This technique is more accurate than microscopy. However, it is expensive, and requires a specialized laboratory (even though technical advances will likely result in field-operated PCR machines).

Treatment of malaria

To treat Malaria we most understand 2 concept:

- The geographic pattern of susceptibility of P. Falciparum to antimalarial drugs.
- The type of plasmodium species causing the infection.



TOXOPLASMOSIS

Toxoplasma gondii

- Worldwide
- Zoonotic parasite; Toxoplasma is an **opportunistic** pathogen.
- Infects animals, cattle, birds, rodents, pigs, and sheep.
- and humans.
- Causes the disease **Toxoplasmosis**.
- Toxoplasmosis is leading cause of abortion in sheep and goats.
- Intracellular parasite.
- Final host (Felidae family, cat)
- Intermediate host (mammals)

Toxoplasmosis

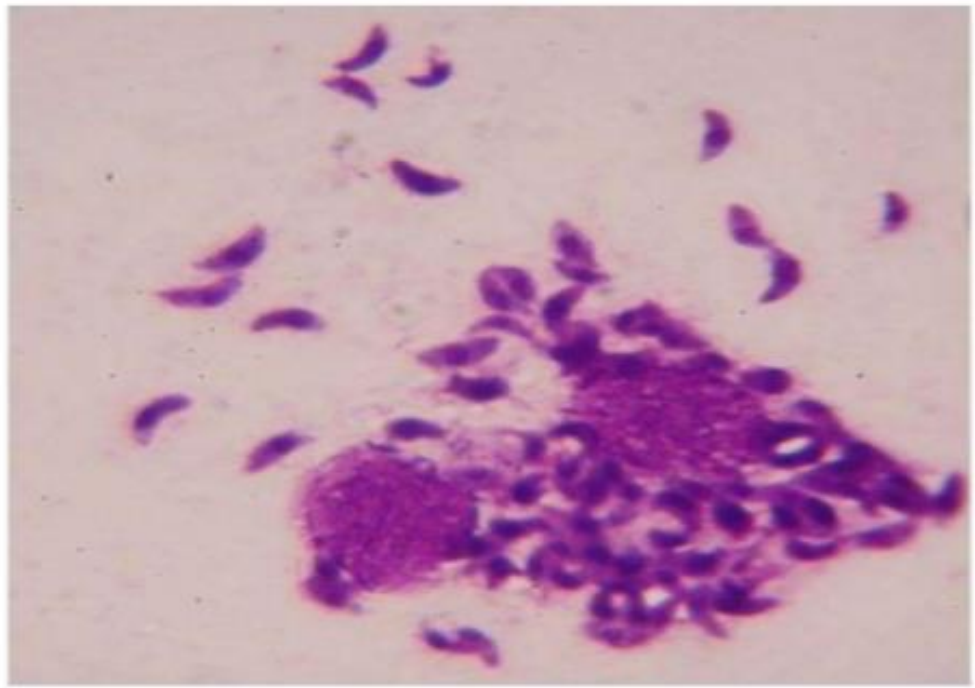
1. **All parasite stages are infectious.**
2. **Risking group:** Pregnant women, meat handlers (food preparation) or anyone who eats the raw meat

Morphology:

Three morphological forms

Asexual forms	**Tachyzoite**
	Bradyzoite (Tissue cyst)
Sexual form	**oocyst**

Image of a tachyzoite



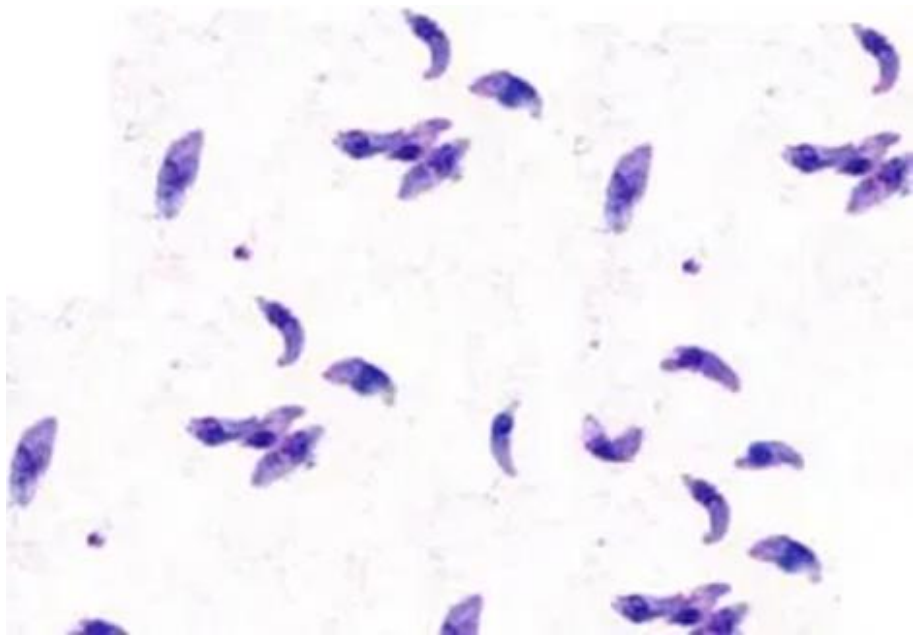
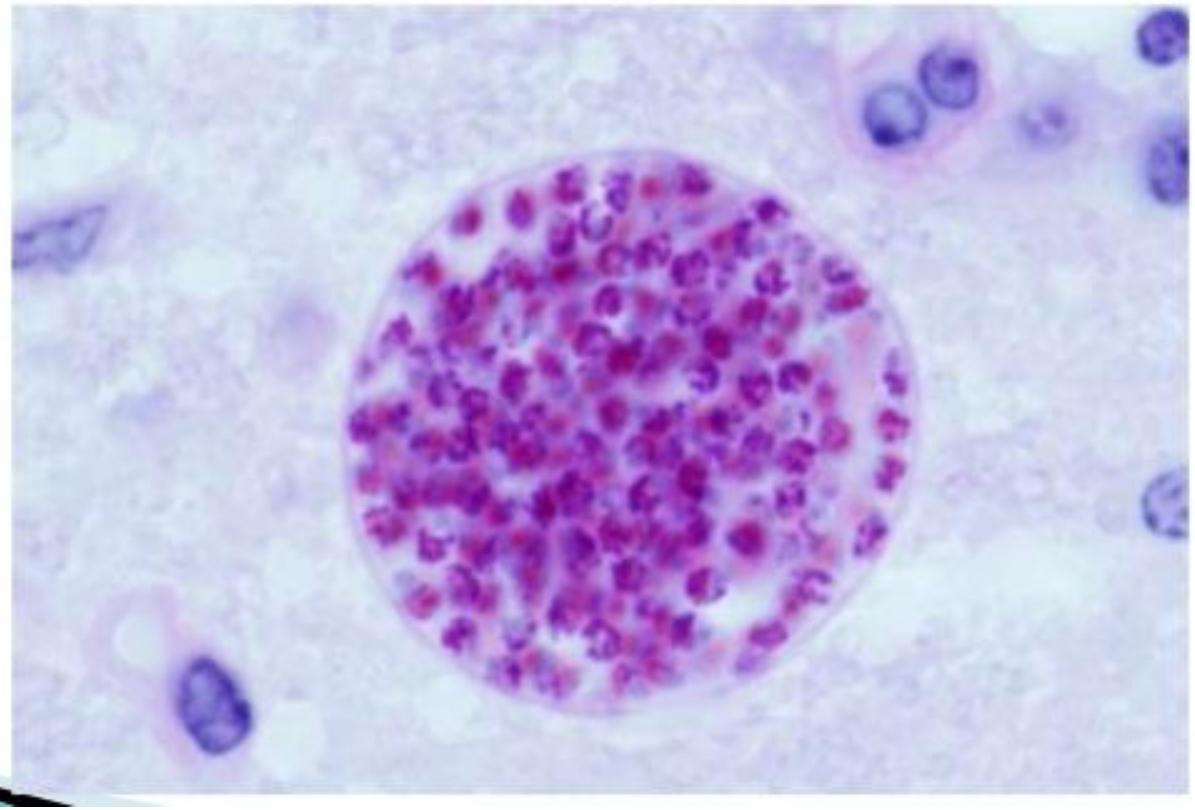
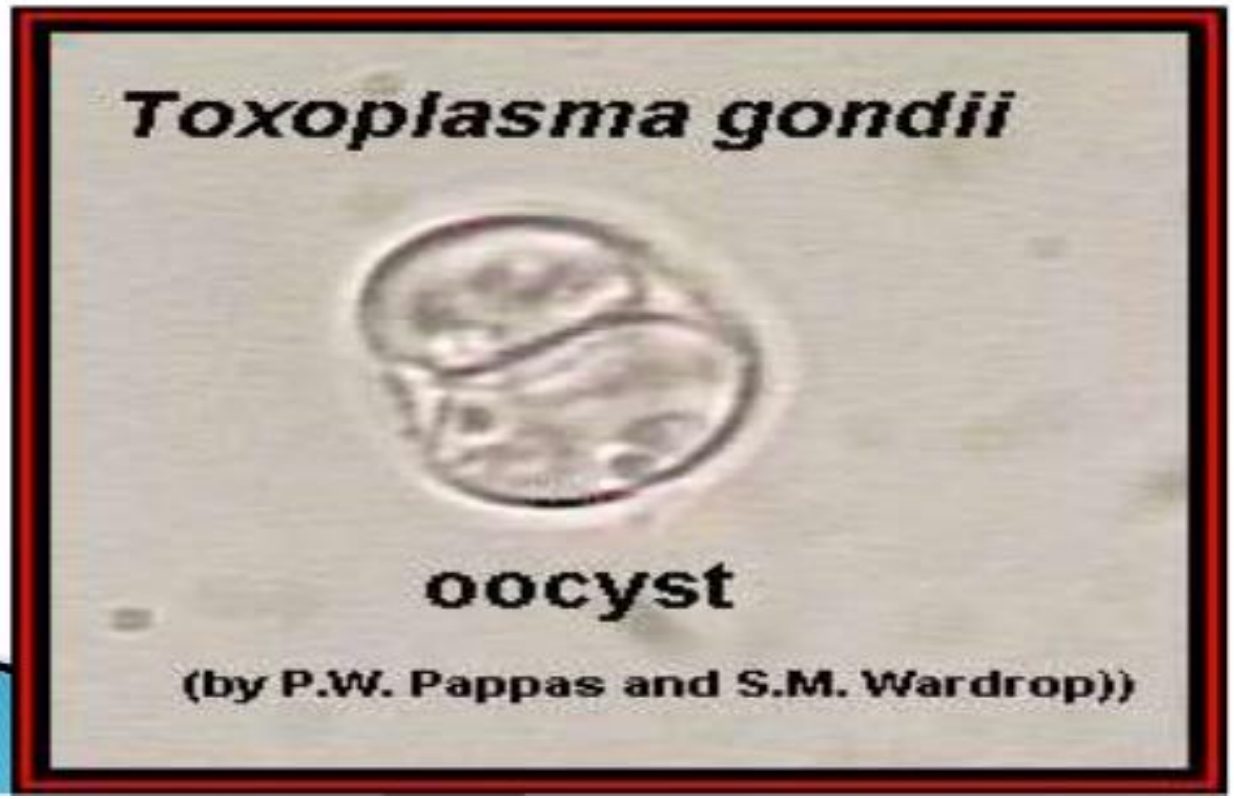


Image of a tissue cyst:



OOCYST:

- Sexual form of the parasite found only in cats and felines



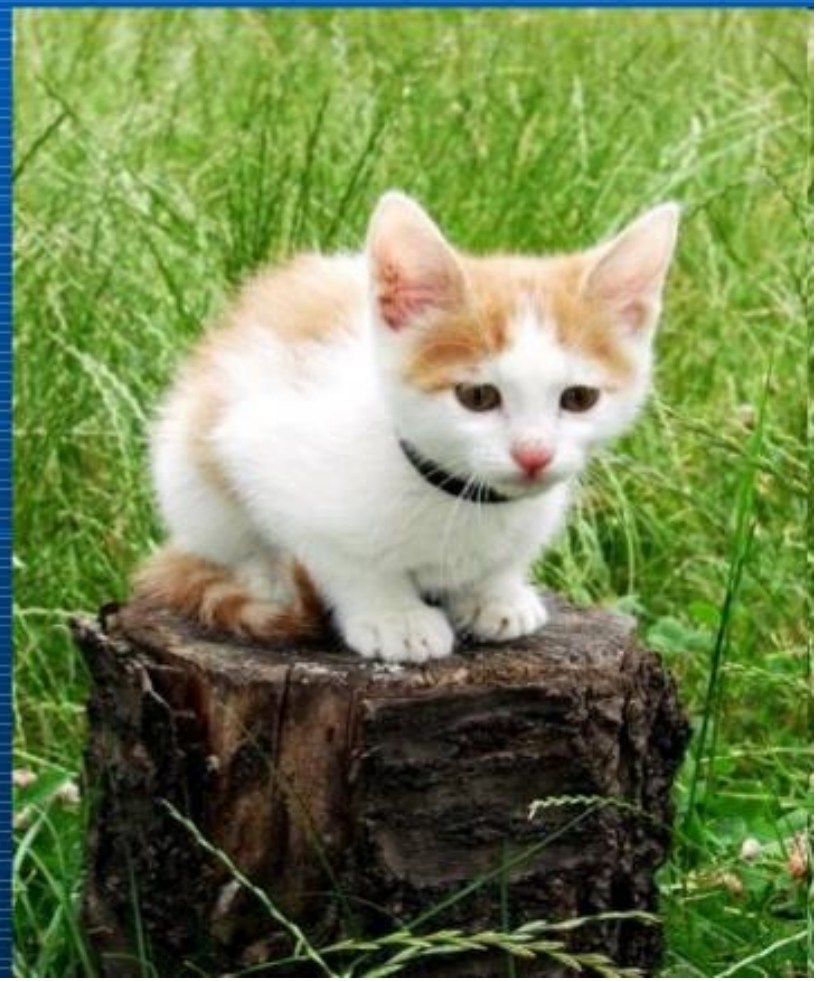
Transmission to man:

1. Ingestion of sporulated oocysts from contaminated soil, food or water
2. Ingestion of tissue cyst containing bradyzoites from undercooked meat
3. By blood transfusion, needle stick injuries, organ transplantation
4. Transplacentral transmission
5. Laboratory accidents

(Tachyzoites are the infective form in blood)

Human Toxoplasmosis

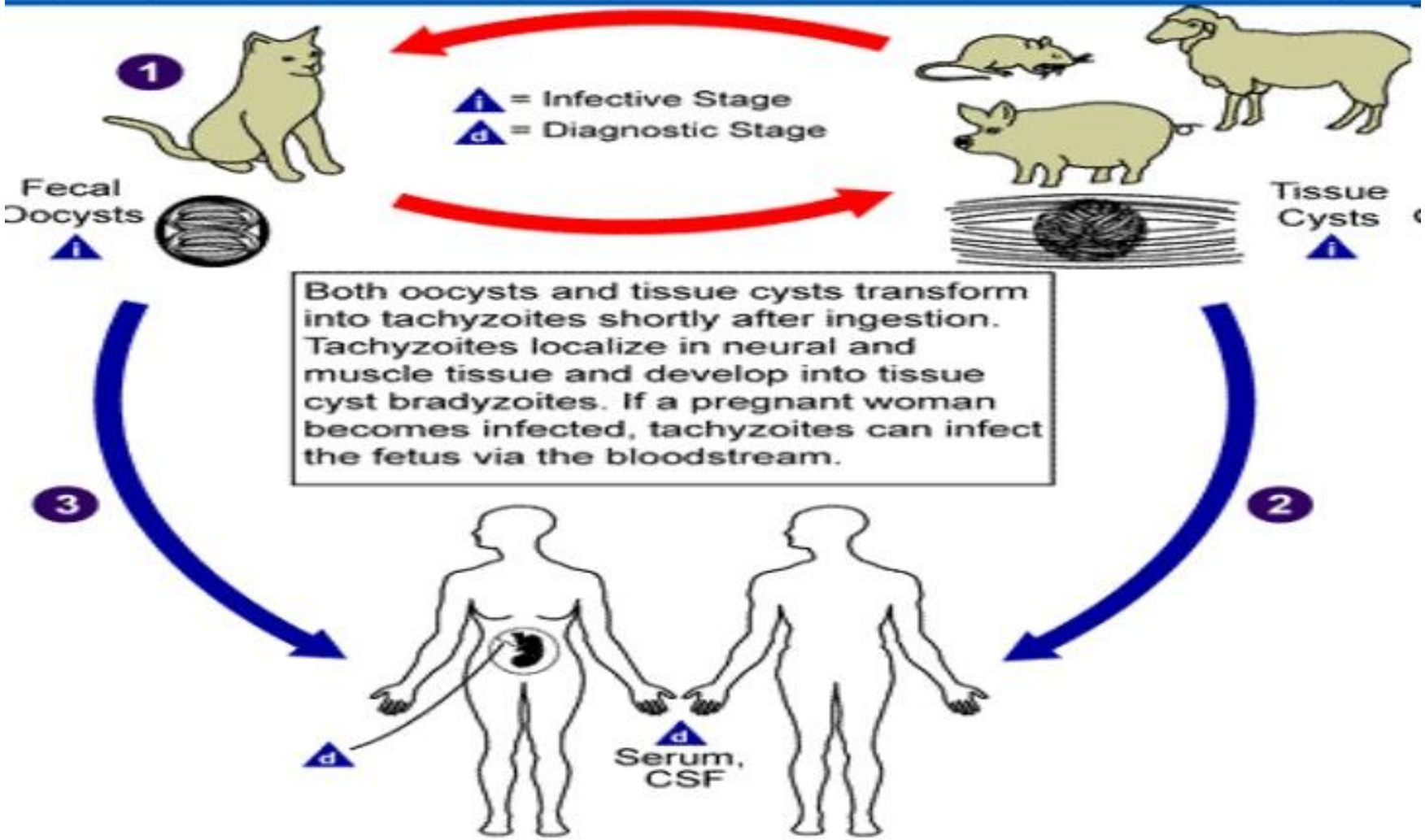
- Toxoplasmosis is a zoonotic disease
- Caused by Coccidian protozoan Toxoplasma gondii
- Infectes a wide range of animals, birds but does not appear to cause disease in them



Toxoplasmosis

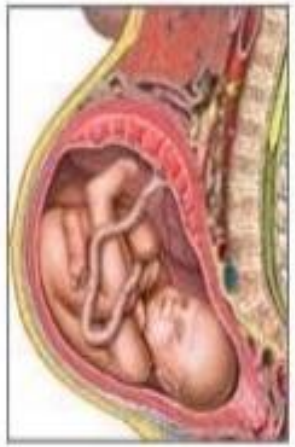
- The normal final host is cat and relatives in the family Felidae, only hosts in which the Oocyst – producing sexual stage of Toxoplasma can develop

Spread of Toxoplasmosis



Implications on Human Health

- In Humans produces
  1 Congenital Toxoplasmosis
  2 Post natal Toxoplasmosis



A fetus may contract toxoplasmosis through the placental connection with its infected mother

The mother may be infected by:

Improper handling of cat litter

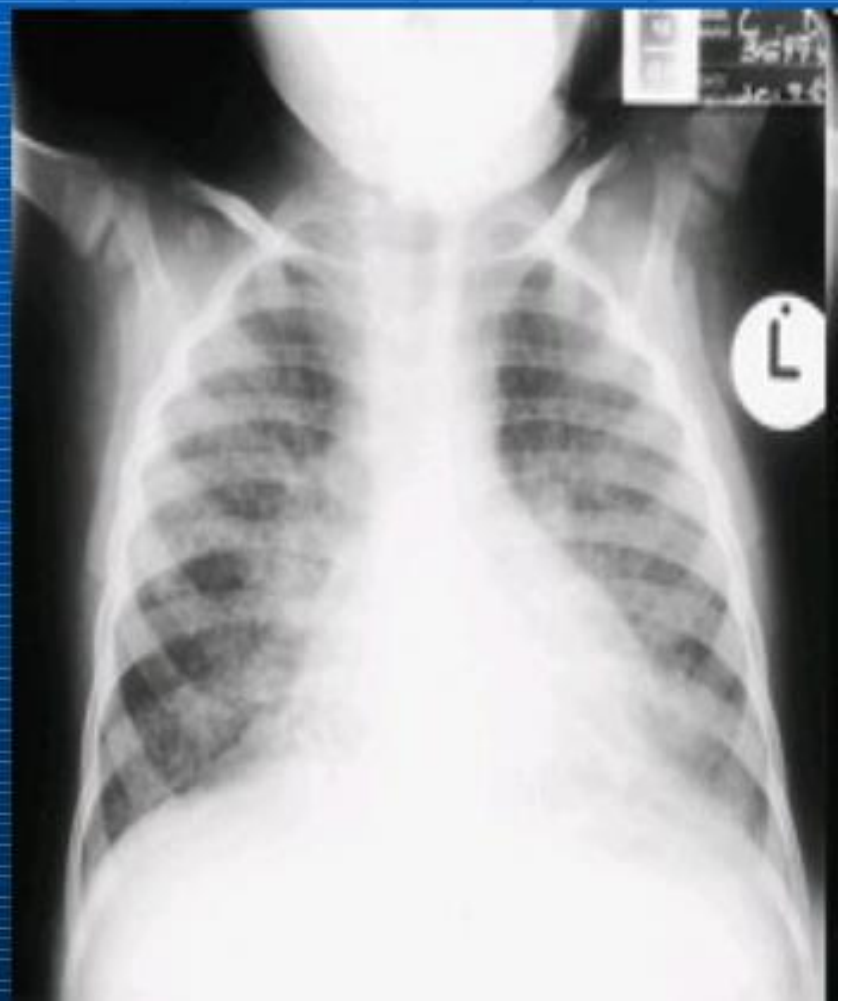
Handling or ingesting contaminated meat

Congenital Toxoplasmosis

- Congenital infection develop in fetus only when non immune mothers are infected during pregnancy
- Post natal Toxoplasmosis is less severe.

Toxoplasmosis - Immunosupressed

- Varying degrees of disease may occur in Immunosupressed indivudals results in
- Retinitis
- Chorioretinits
- Pneumonias
- Other non specific manifestions



Leishmania

INTRODUCTION

- ***Leishmania***:
  - Is a genus of trypanosomatid protozoa, which causes a fatal vector-borne parasitic disease called Leishmaniasis.
  - It is spread by the bite of sandflies of the genus *Phlebotomus* in the Old World, and of the genus *Lutzomyia* in the New World.

- **Leishmaniasis**:
  - is the second-largest parasitic killer in the world (after malaria) and is endemic in many parts of Africa, Asia and South America.

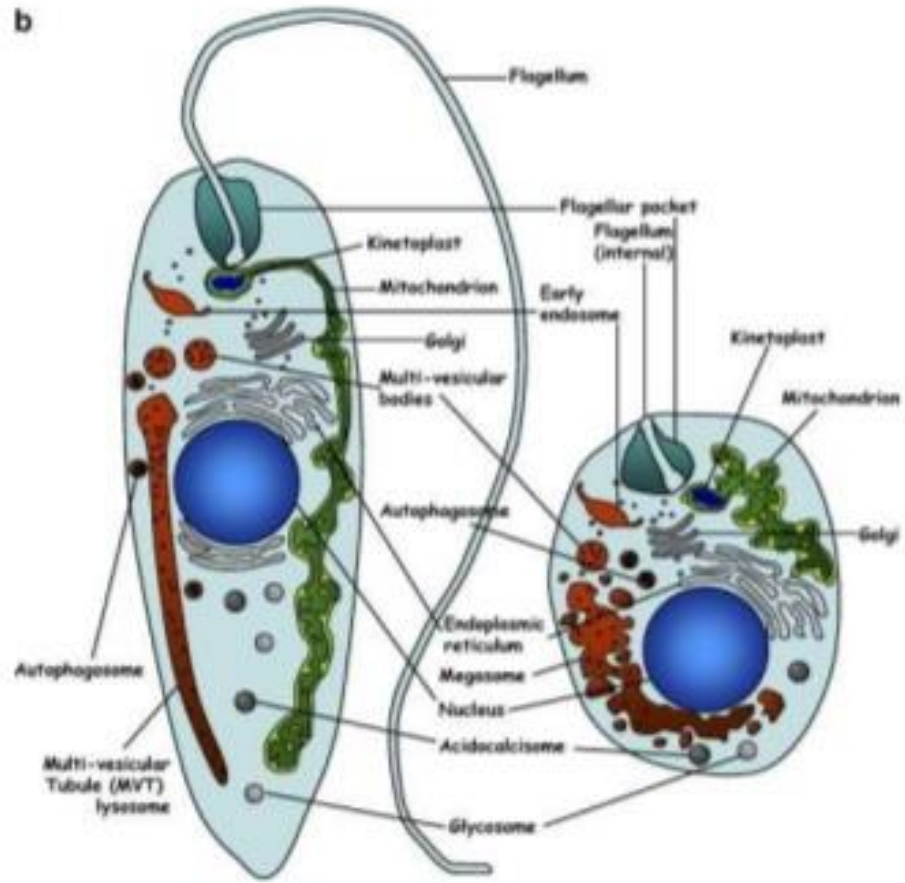
MORPHOLOGY

(same in all species)

- The parasite exists in 2 forms;-

1. Amastigotes – aflagellar stage

2. Promastigotes- flagellar stage



Morphological Differences

Amastigotes

- Aflagellar stage
- Occurs in the vertebrate host
- divides by binary fission at 37°C.
- There are round or oval ;2-4µm along longitudinal axis.
- Nucleus relatively larger and situated centrally.

Promastigotes

- Flagellar stage
- Occurs in the sand fly
- divides by binary fission at 27°C.
- They are spindle shaped ;15-20 µm in length & 1-2µm in width.
- Nucleus smaller and situated in the middle of the cell or along the side of cell-wall.

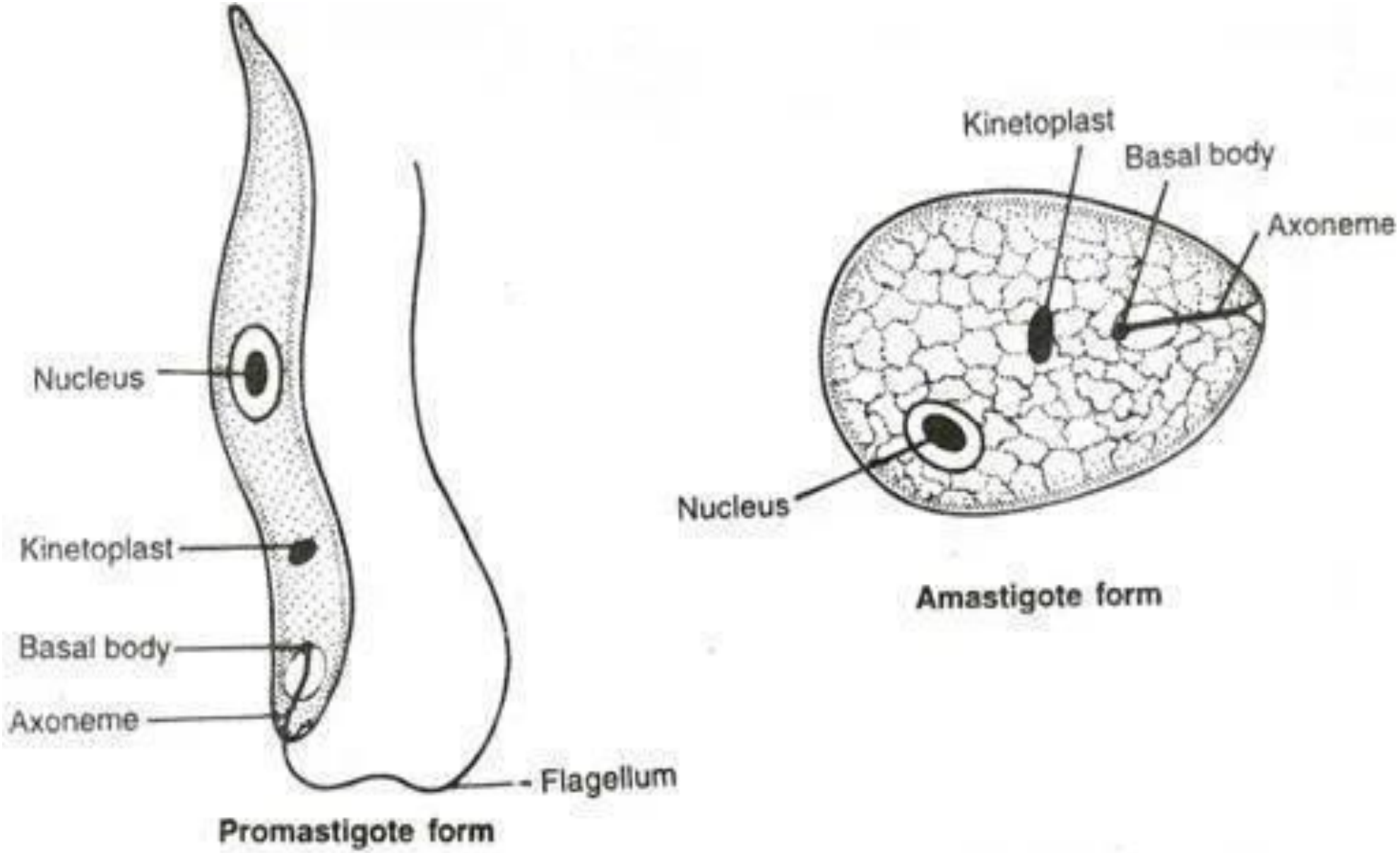
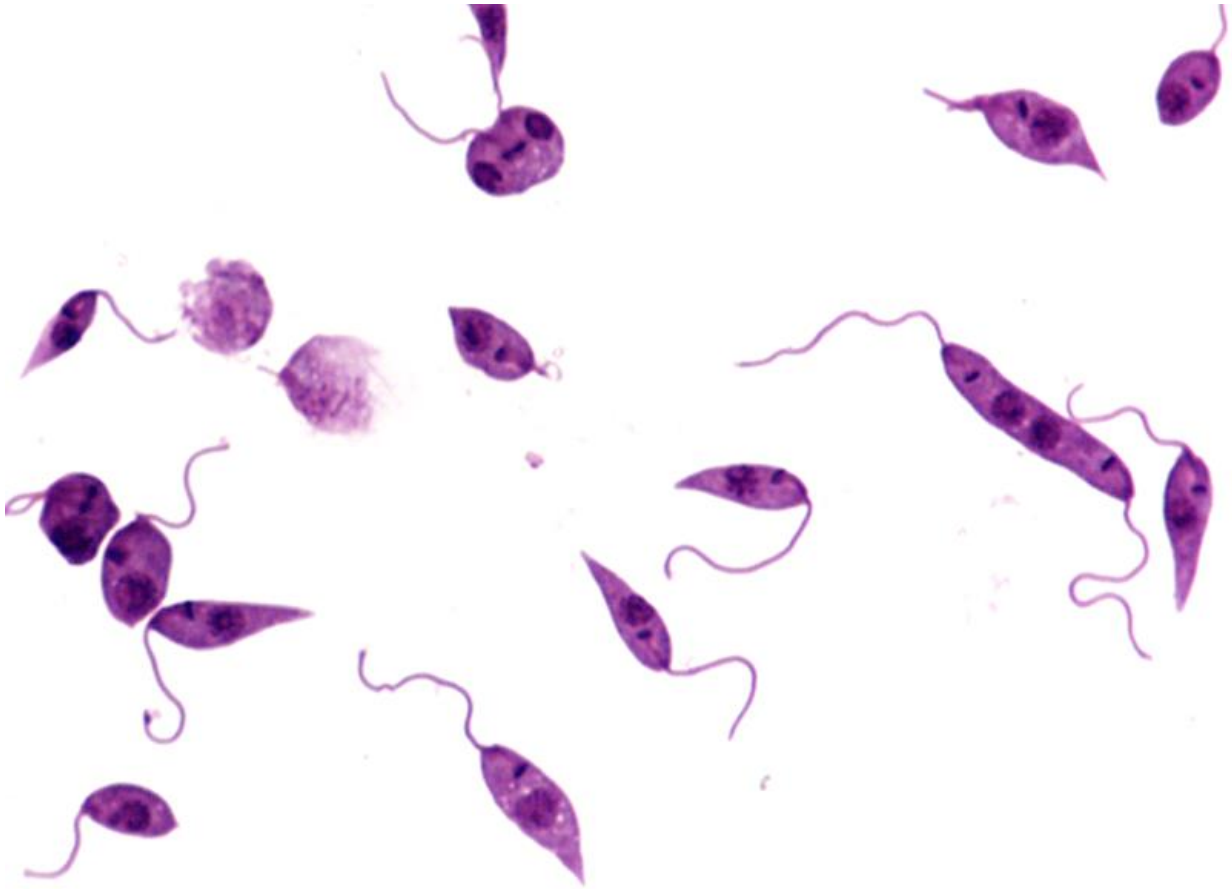


Fig. 178. Morphological forms of *Leishmania donovani*



Leishmania donovani

IMPORTANT SPECIES

- *L. donovani*
- *L. tropica*
- *L. mexicana*
- *L. braziliensis*
- *L.major*
- *L.guyanensis*
- *L.lainsoni, etc*

MODE OF TRAMSMISSION

(*L.donovani*)

1. Mainly by the bite of sand fly (vector) Phlebotomus argentipus
2. **Less frequently by:**

- blood transfusion,
- congenital infection,
- accidental inoculation of cultured promastigotes in the lab. Workers.
- sexual intercourse.

- ❑ Males are affected more (due to increased exposure to sand flies through the occupation and leisure activities).

VECTOR AND HOST

VECTOR

(Sand fly)

- *Phlebotomas*
- *Lutzomyia*

CLINICAL MANIFESTATIONS

1. Fever
2. Spleen enlargement
3. Lymphadenopathy
4. Darkening of the skin (***KALA AZAR*, MEANING "BLACK FEVER)**

- Complications:- pneumonia, TB, dysentery, uncontrolled haemorrhage
- Prognosis:- With an early treatment, cure rate >90%

  If not treated, death occurs within 2 years.

TYPES OF LEISHMANIASIS

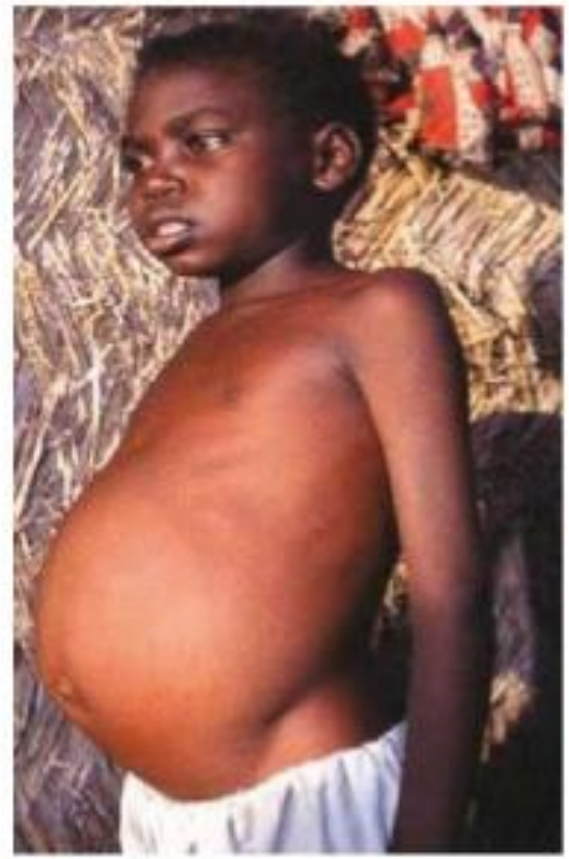
Leishmaniasis is divided into clinical syndromes according to what part of the body is affected most.

- Visceral Leishmaniasis(VL)
- Cutaneous Leishmaniasis(CL)
- Mucocutaneous leishmaniasis(MCL)

1. Visceral Leishmaniasis (VL) or Kala-azar

- caused by *L.donovani*

- part of the body affected most is internal organs

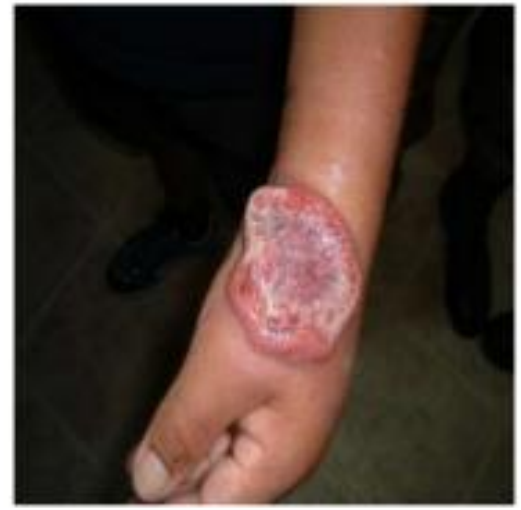


Spleenomegaly

Continued....

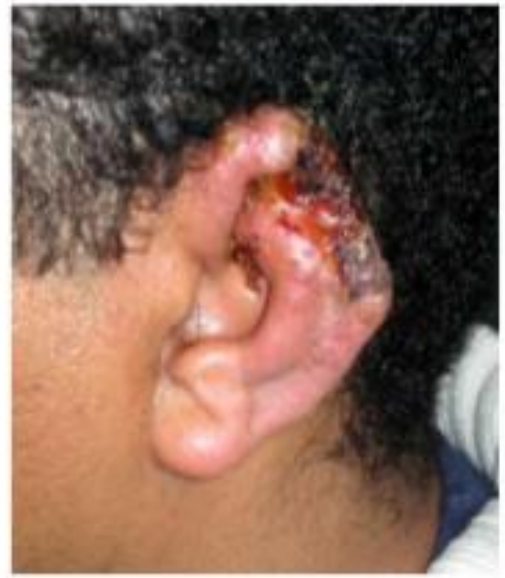
2. Cutaneous Leishmaniasis(CL)

(most common type)



a) **Old world CL:-** caused by ***L.tropica, L. aethiopica***

b) **New world CL:-** caused by *L.mexicana, L.braziliensis, L.guyanensis*

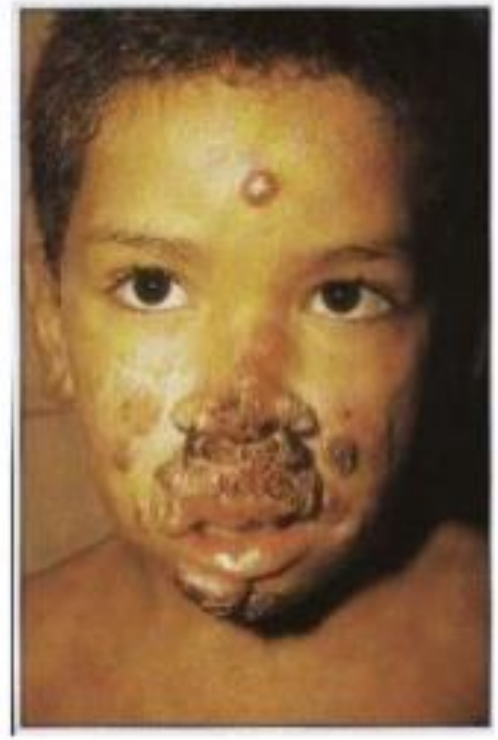


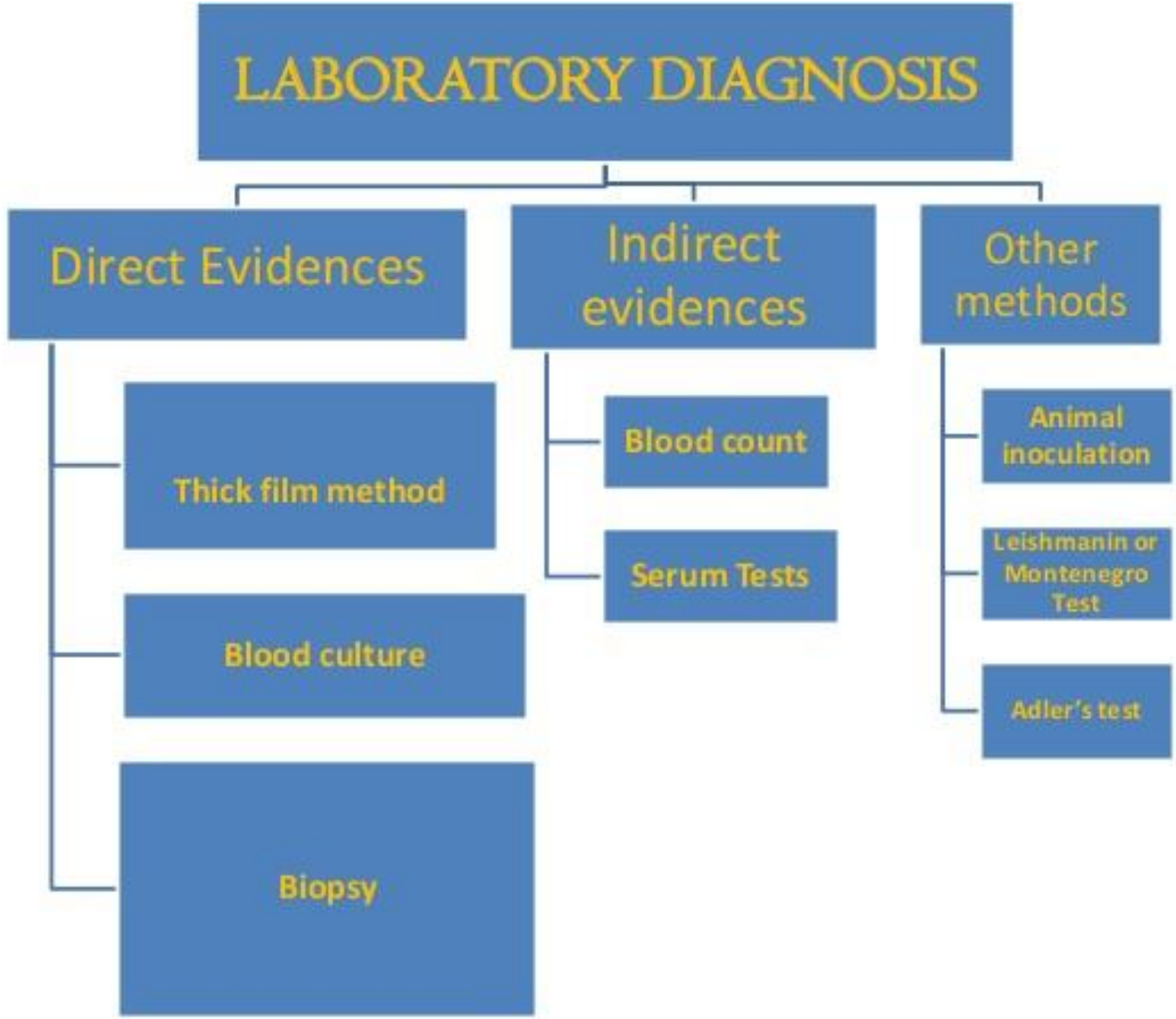
c) **Dermal leishmanoid** or **Post kala-azar dermal leishmaniasis(PKDL):-** caused by *L.donovani*

- Part of the body most affected is **skin**

3. Mucocutaneous leishmaniasis(MCL)

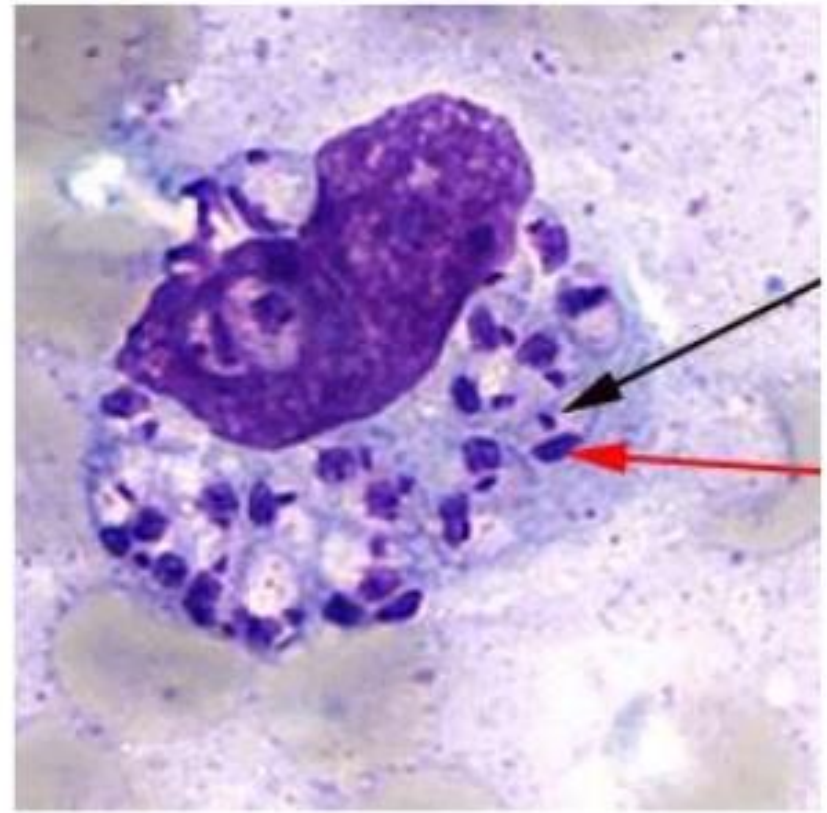
- Caused by *L. braziliensis* and occasionally by *L.panamensis*
- Part of the body affected most is skin and mucous membrane of nose and pharynx




LABORATORY DIAGNOSIS
Direct Evidences
Indirect evidences
Other methods
Thick film method
Blood culture
Biopsy
Blood count
Serum Tests
Animal inoculation
Leishmanin or Montenegro Test
Adler's test

Direct Evidences (contd.....)

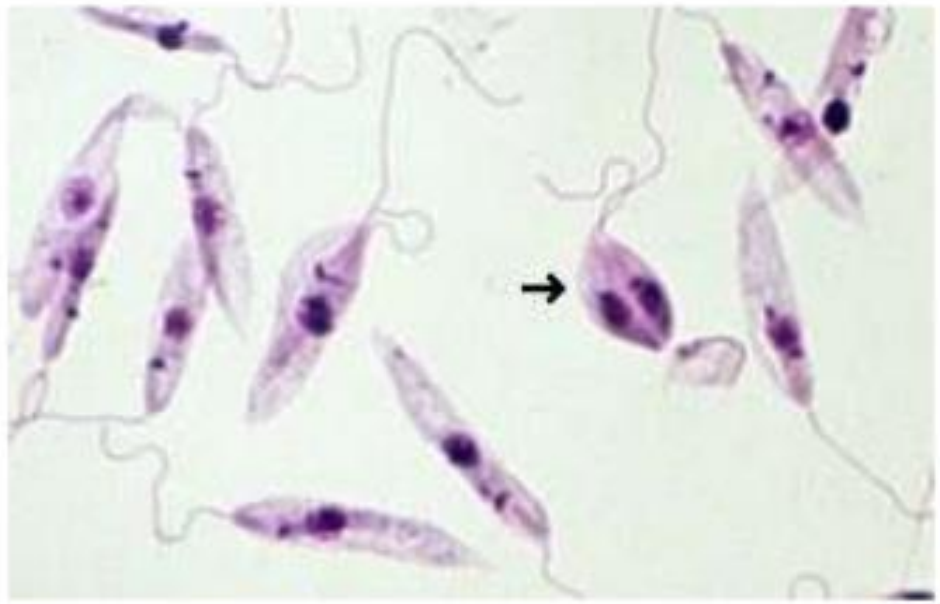
1. **Peripheral blood by thick film method.(Amastigote form)**



Amastigotes in a macrophage

Direct Evidences (contd.....)

2. **Blood culture** in N.N.N. Medium. (Promastigote form)



Promastigote from culture in NNN medium

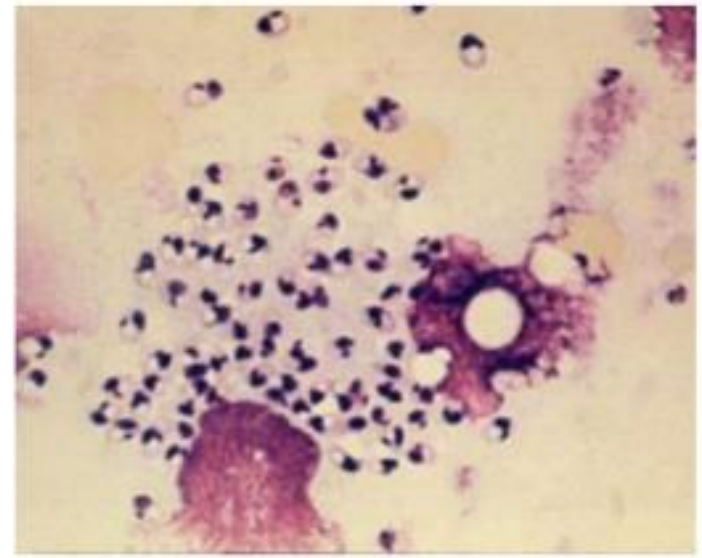
Direct Evidences (....contd)

3. **Biopsy** material obtained by

- lymph node puncture,
- sternal or iliac crest puncture(marrow) and
- spleen puncture(spleen pulp)

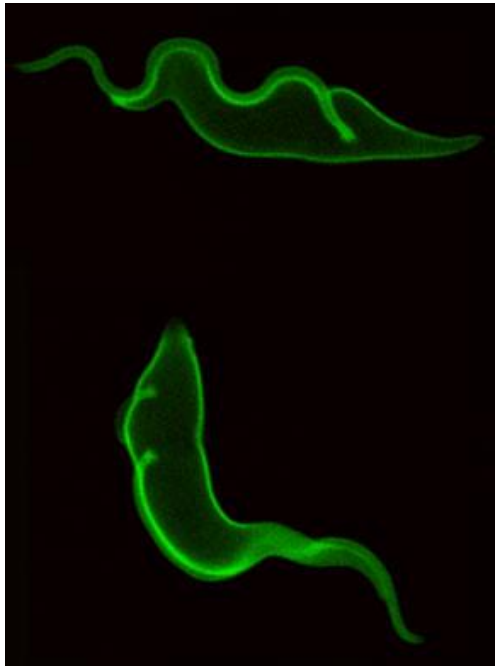
Amastigote form in a stained smear

Promastigote in culture in NNN medium



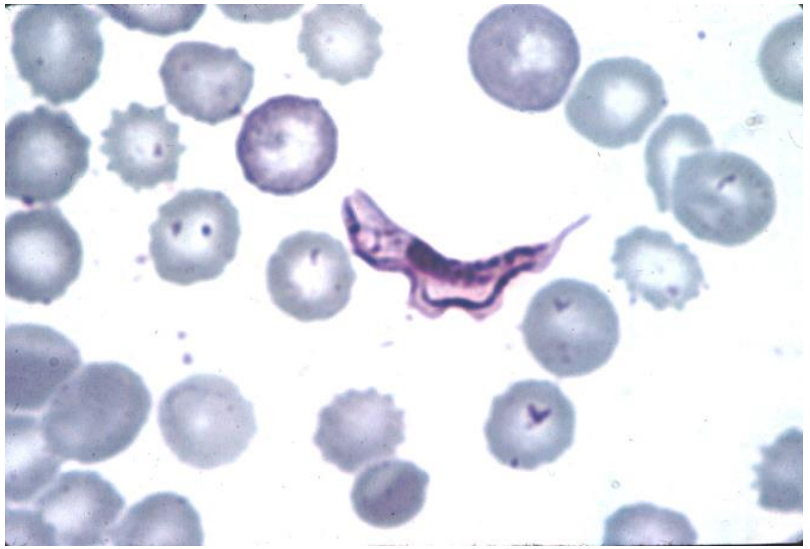
Amastigotes of *L. donovani.*
Splenic aspirate.

Trypanosomiasis.
Sleeping sickness.



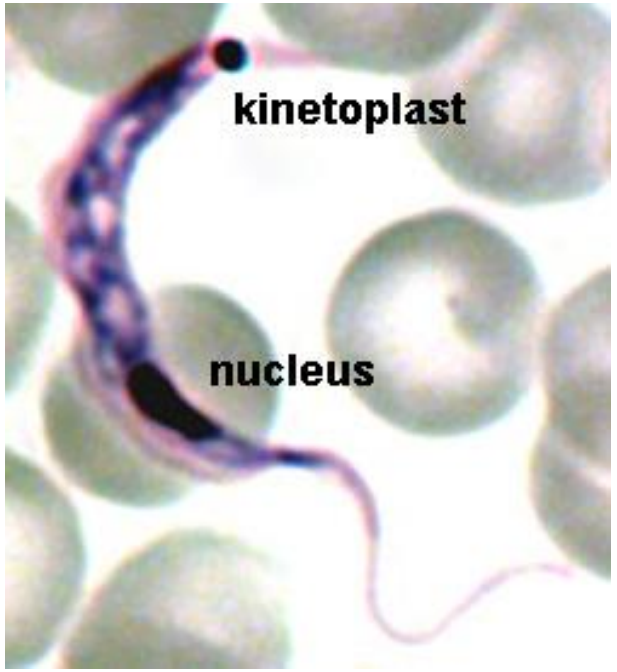
African trypanosomiasis (Sleeping sickness)

- – protozoan disease transmitted to human beings by the bite of infected tsetse flies.



Classification:

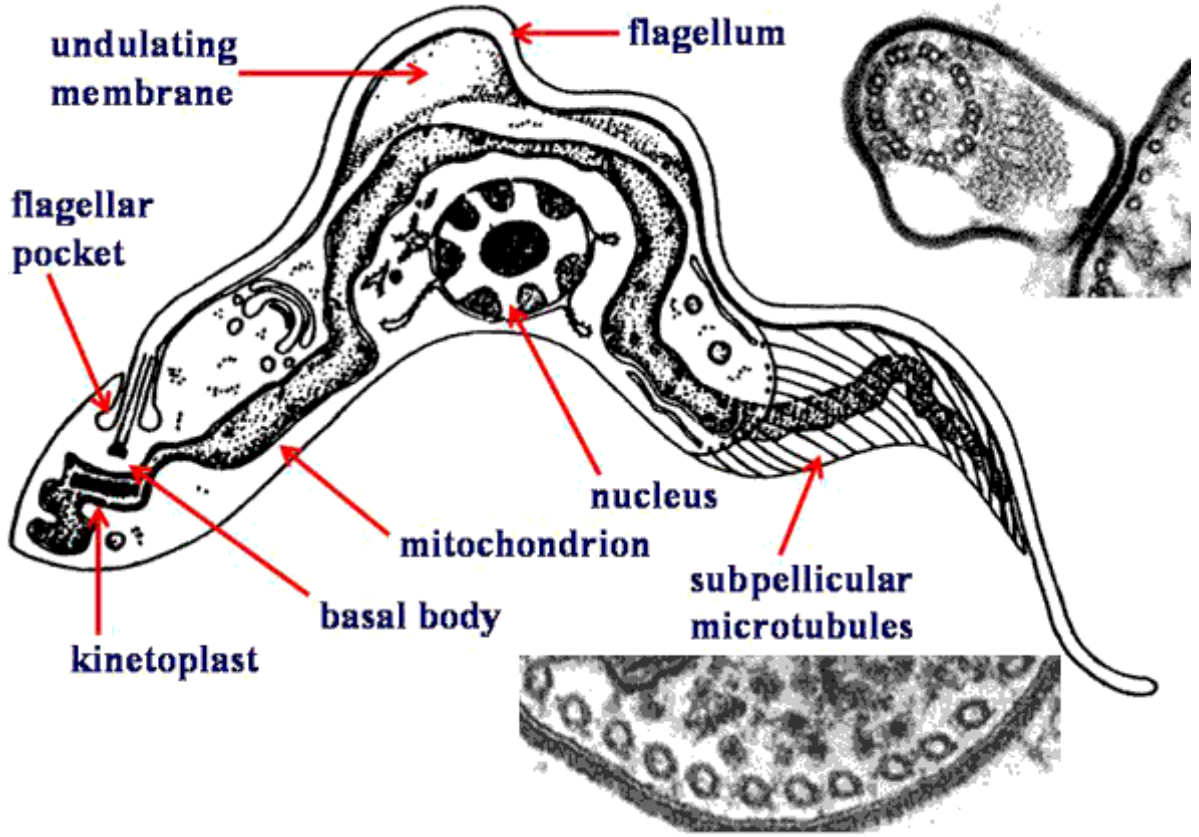
- Eukaryota (organisms with nucleated cells),Kingdom Protista, Phylum Protozoa.
- East African trypanosomiasis is caused by the parasite Trypanosoma brucei rhodesiense.
- West African trypanosomiasis is caused by Trypanosoma brucei gambiense. The parasites are spread by tsetse flies, found only in Africa.

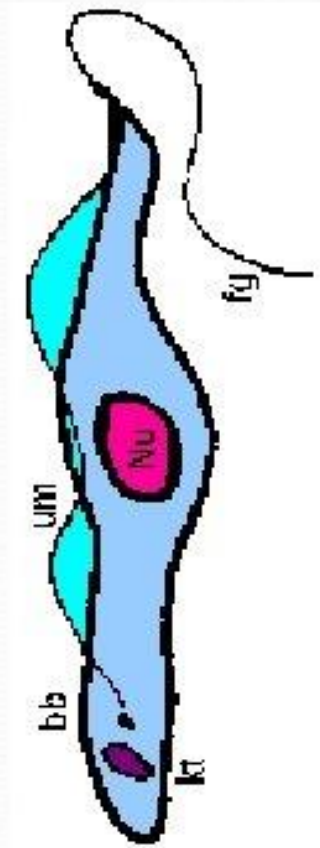
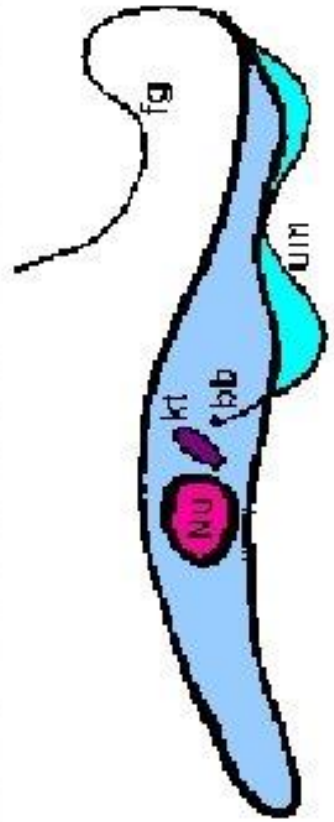
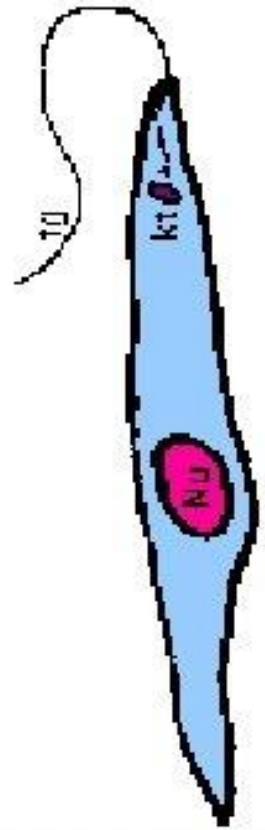
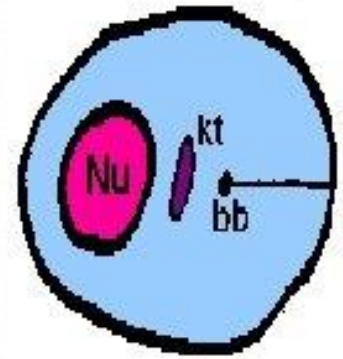


Morphology

Trypanosomes have a single central nucleus and a single flagellum originating at the kinetoplast and joined to the body by an undulating membrane. The outer surface of the organism is densely coated with a layer of glycoprotein, the variable surface glycoprotein (VSG).

From the point of view of functional and physiologic complexity, a protozoan is more like an animal than like a single cell.

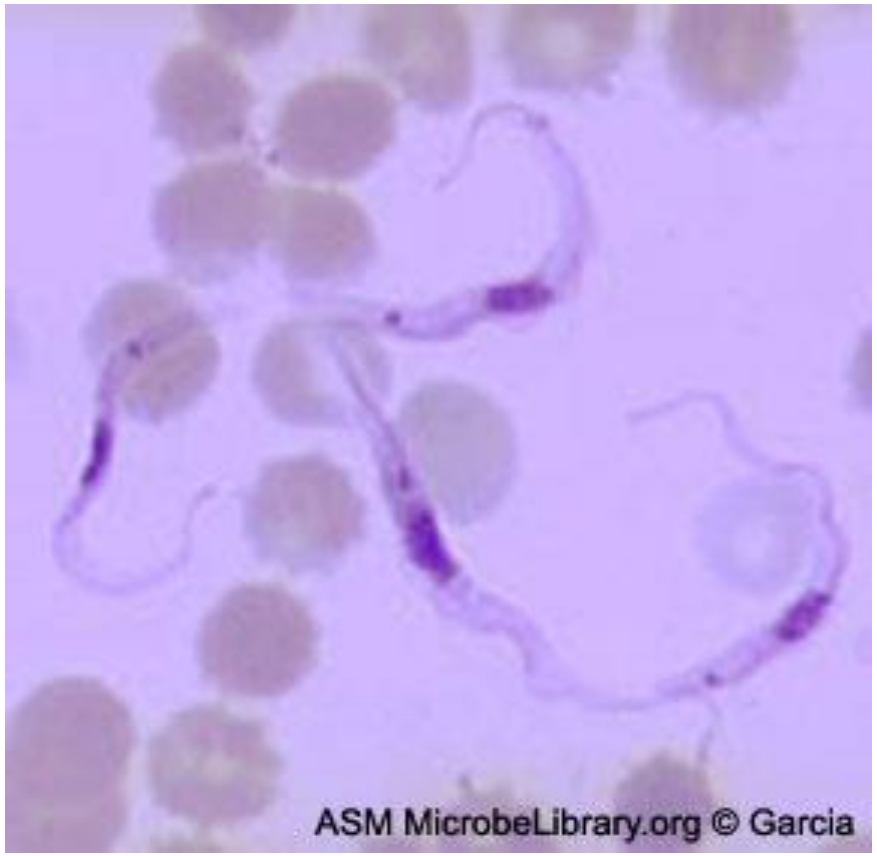


	Trypomastigote	Epimastigote	Promastigote	Amastigote
FORM				

Etiology

There are two clinical forms of African trypanosomiasis:

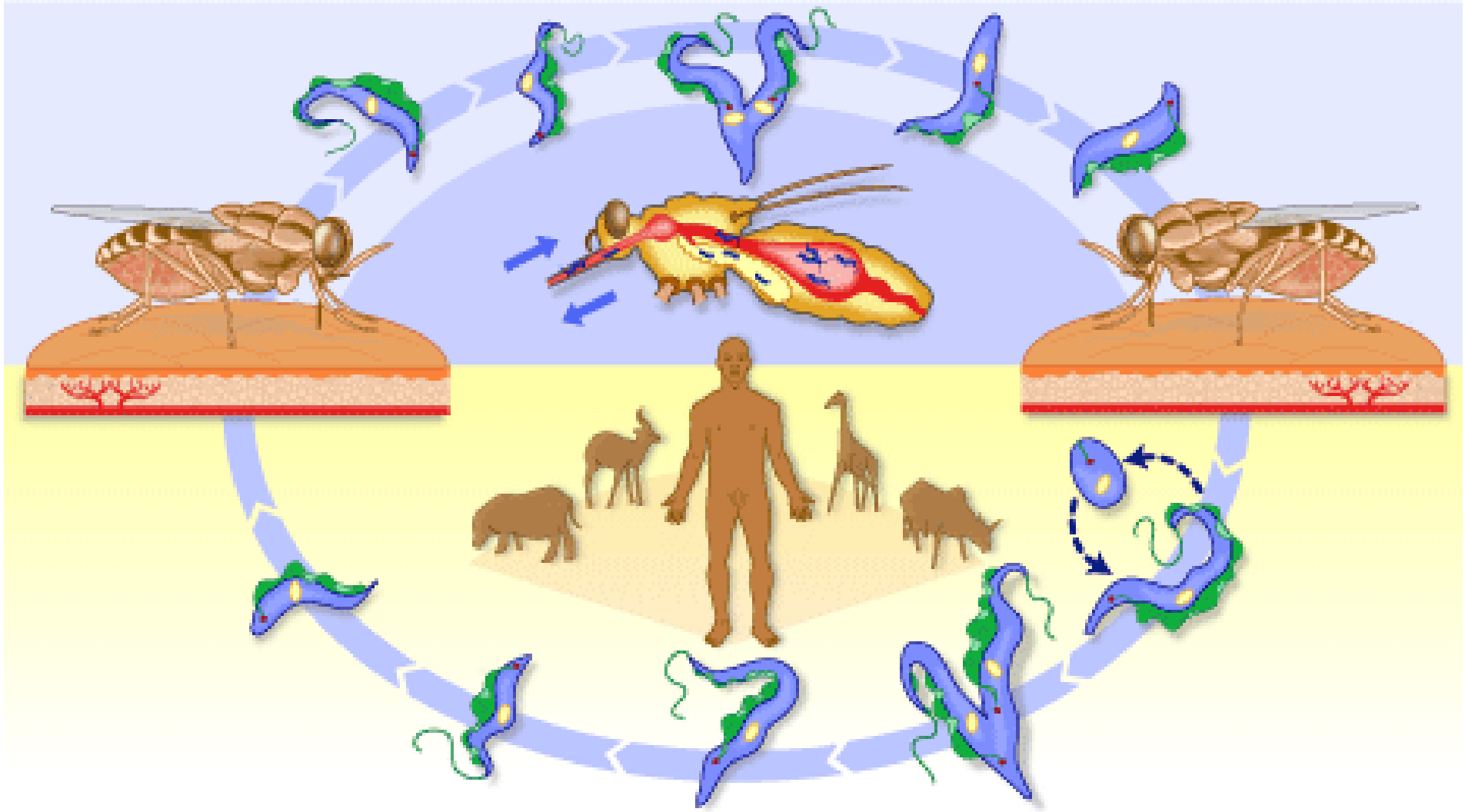
- 1) a slowly developing disease caused by *Trypanosome brucei gambiense* and
- 2) a rapidly progressing disease caused by *T. brucei rhodesiense.*



Major Differences Between African Trypanosome Species

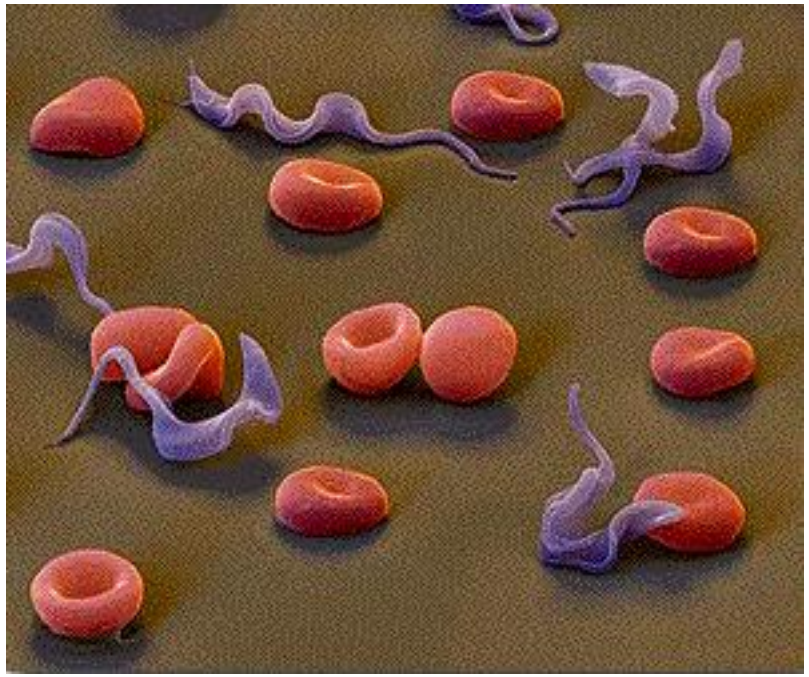
Attribute	***T. rhodesiense***	***T. gambiense***
tsetse vector	*G. morsitans* group	*G. palpalis* group
ecology	dry bush, woodland	rainforest, riverine, lakes
transmission cycle	ungulate-fly-human	human-fly-human
non-human reservoir	wild animals	domestic animals
epidemiology	sporadic, safaris	endemic, some epidemics
disease progression	rapid, often fatal	slow (~1 yr) acute ⇒ chronic
parasitemia	high	low
asymptomatic carriers	rare	common

Life cycle of Trypanosoma brucei parasites



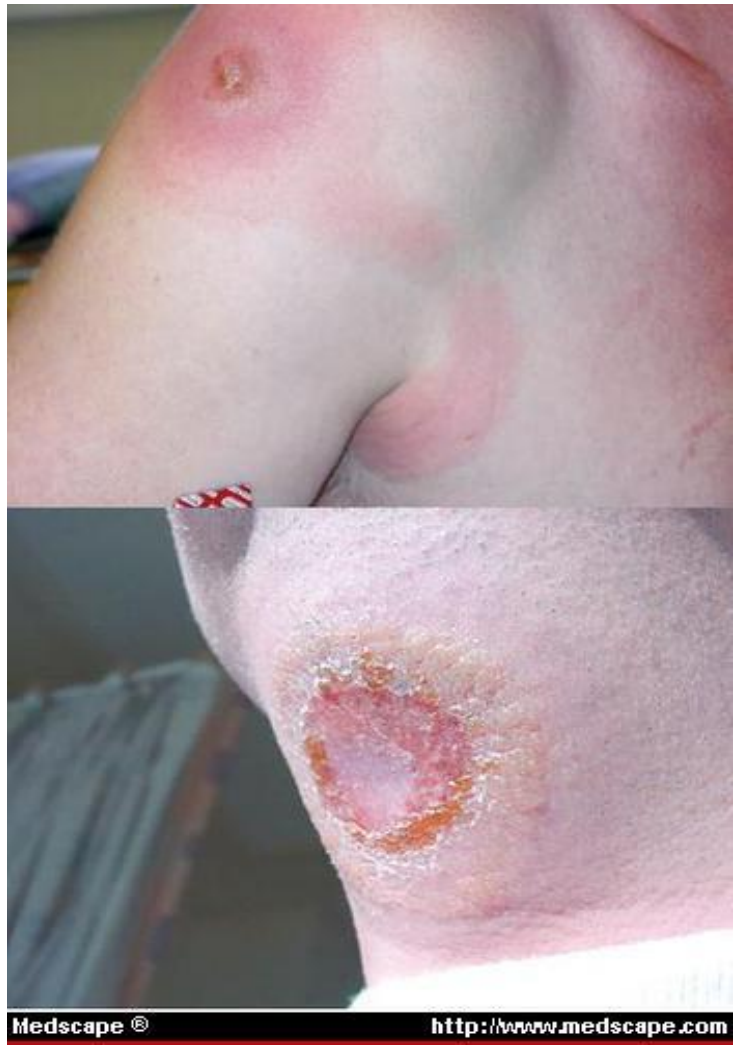
Pathogenesis

- Inflammatory changes (possibly autoimmune) cause CNS demyelination. Immunosuppression by the parasite facilitates secondary infections.



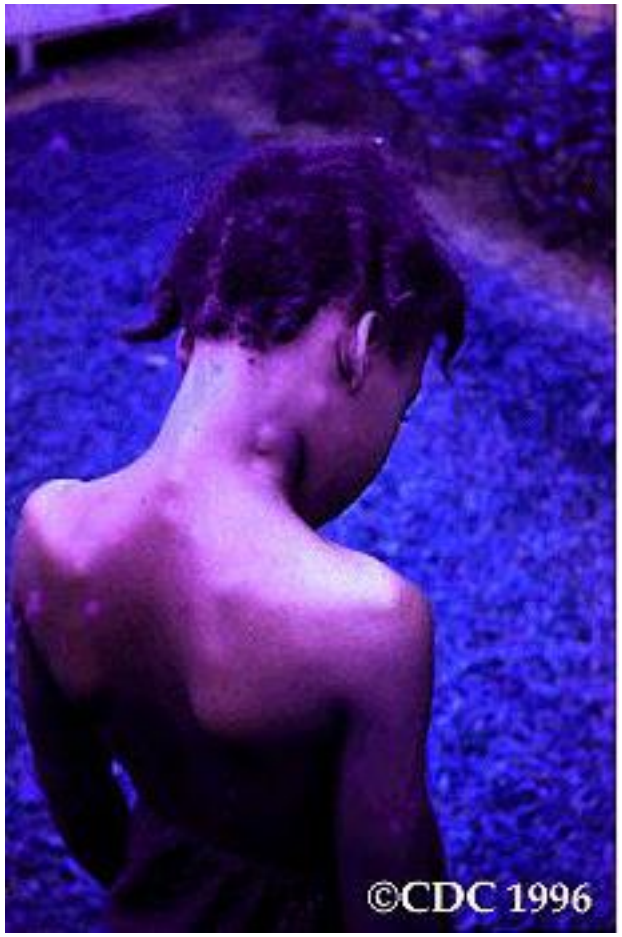
Clinical Symptoms

- A tsetse fly bite is often painful and can develop into a red sore, called a chancre.



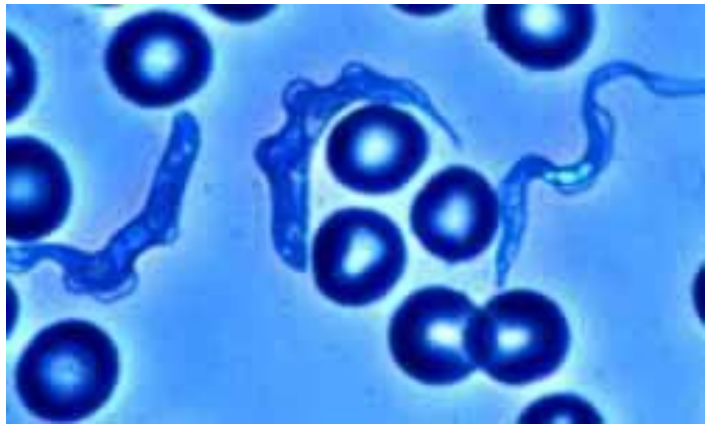
Clinical Symptoms

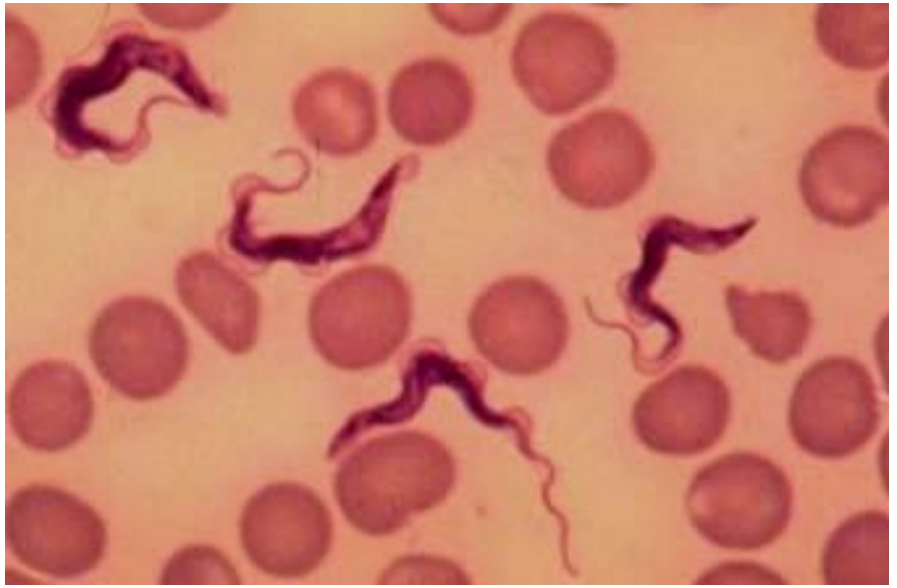
- fever,
- severe headache,
- irritability,
- extreme tiredness,
- swollen lymph glands,
- aching muscles and joints.



Laboratory Diagnostics:

- In the early stages of the disease, the parasites can be demonstrated in lymph nodes and blood; later, they appear in the cerebrospinal fluid. In the Rhodesian type, lumbar puncture is indicated because of early CNS invasion.
- Culture or laboratory animal inoculations can be useful.
- Serologic tests, such as indirect immunofluorescence, direct card agglutination, and indirect hemagglutination, are used successfully for diagnosis.





Treatment

- Medicine for the treatment of African trypanosomiasis is available. Treatment should be started as soon as possible and is based on the infected person's symptoms and laboratory tests results.
- Patients need to be hospitalized for treatment and require periodic follow-up exams for 2 years.
- The current standard treatment for first stage disease is: Intravenous pentamidine (for T.b. gambiense); or suramin (for T.b. rhodesiense).
- The current standard treatment for second stage disease is: Intravenous melarsoprol.
- In areas with melarsoprol resistance or in patients who have relapsed after melarsoprol monotherapy, the treatment should be: melarsopsol and nifurtimox, or eflornithine.

Prevention

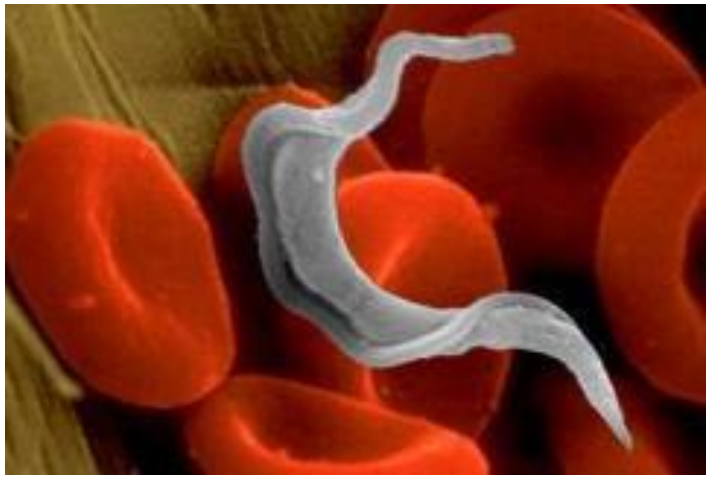
- There is no vaccine or drug to prevent African trypanosomiasis.
- When traveling in areas where the disease occurs, take these precautions against bites from tsetse flies and other insects.

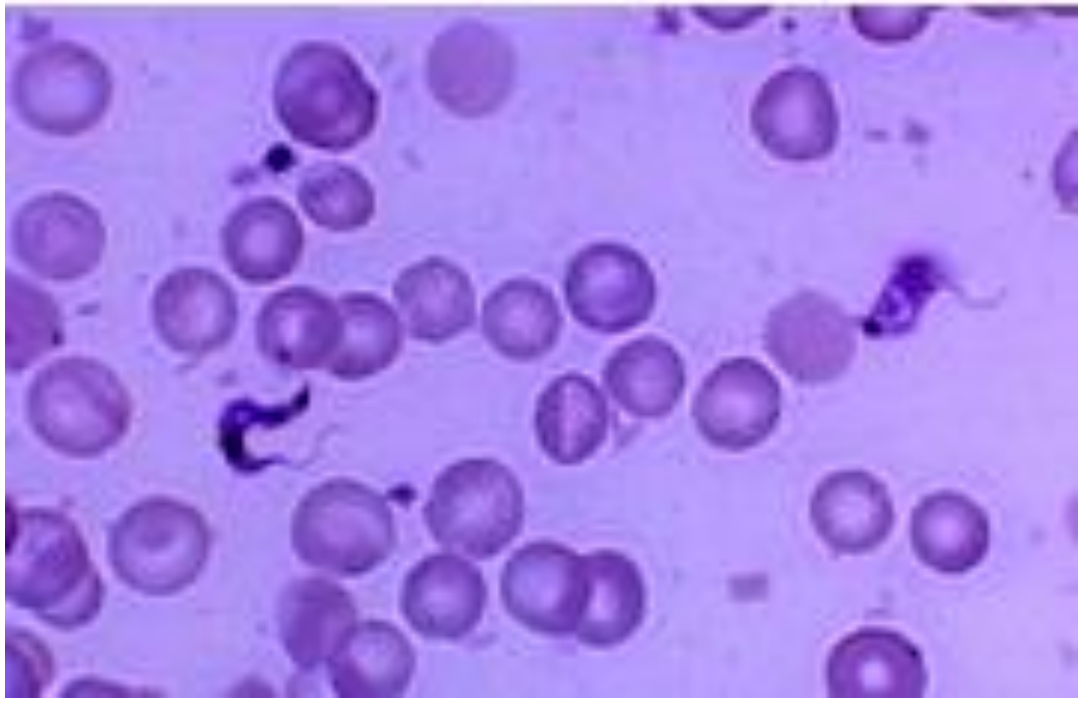
American Trypanosomiasis (Chagas disease)

- ***Trypanosoma cruzi*** belongs to the subkingdom Protozoa. They are flagellar organisms that have one nucleus and an organelle, the kinetoplast, that gives rise to one mitochondrion and mitochondrial DNA.

Classification

- Eukaryota (organisms with nucleated cells), Kingdom Protista, Phylum Protozoa.
- *Trypanosoma cruzi.*
- *T. cruzi* reproduce asexually by binary fission.
- Like all other trypanosomes, *T. cruzi* live one stage of their lives in the blood and/or tissues of vertebrate hosts and during other stages they live in the digestive tracts of invertebrate vectors (temporary hosts).





- Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in new infection sites.
- The bloodstream trypomastigotes do not replicate.
- Replication resumes only when the parasites enter another cell or are ingested by another vector.
- The “kissing” bug becomes infected by feeding on human or animal blood that contains circulating parasites.
- The ingested trypomastigotes transform into epimastigotes in the vector’s midgut .
- The parasites multiply and differentiate in the midgut.

Epidemiology

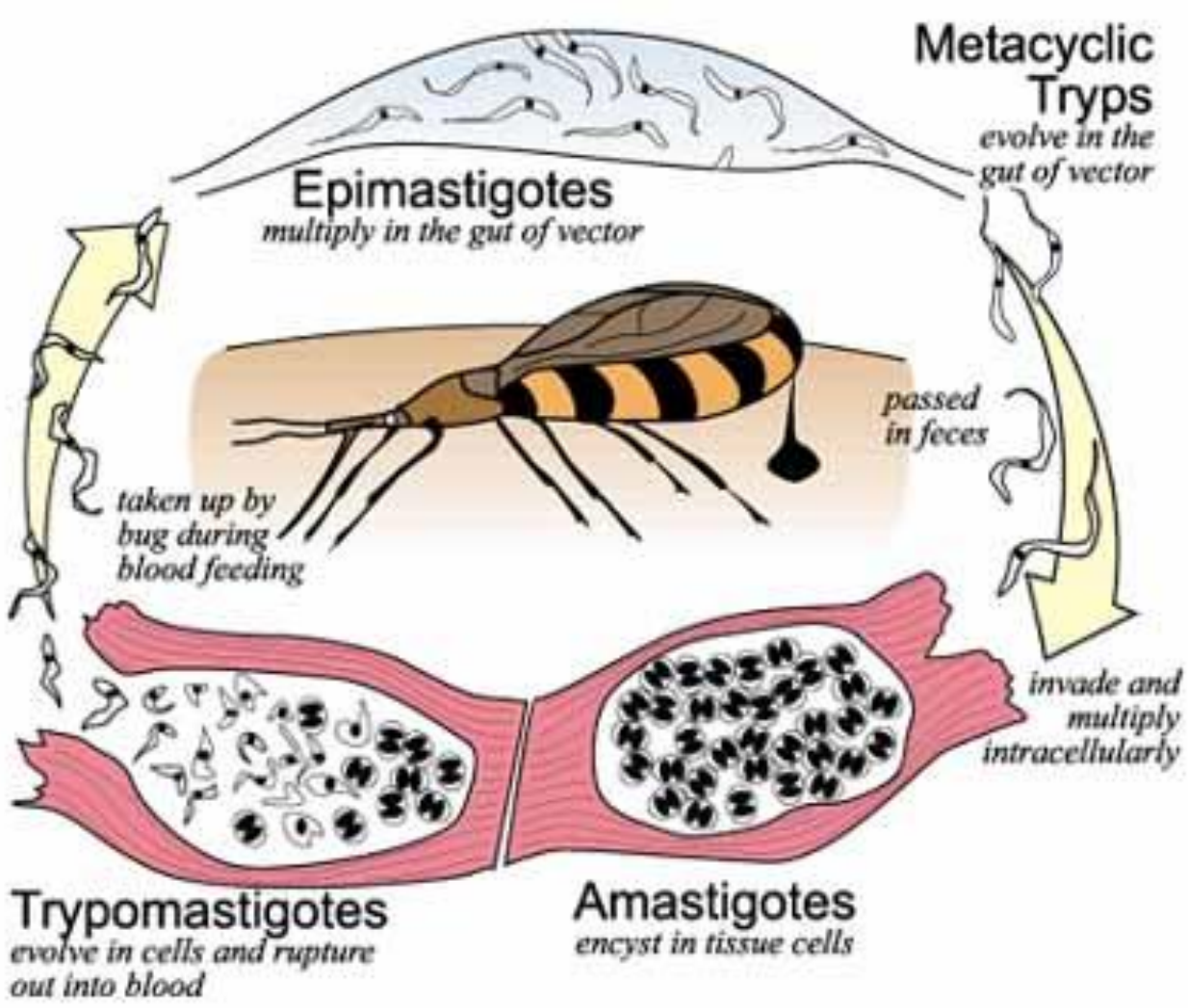
- Chagas disease is transmitted by cone-nosed triatomine bugs of several genera (Triatoma, Rhodnius, Panstrongylus).
- **Trypanosoma cruzi can also be transmitted through blood transfusions, organ transplantation, transplacentally, breast milk and in laboratory accidents.**

Adult *Rhodnius prolixus*, a kissing bug.
WHO/TDR/Stammers

Transmission methods of T. cruzi

- ***Contamination***

Contamination through the insect's feces is the primary mechanism by which *vinchucas* pass *T.cruzi* to humans.



Transmission methods of T. cruzi

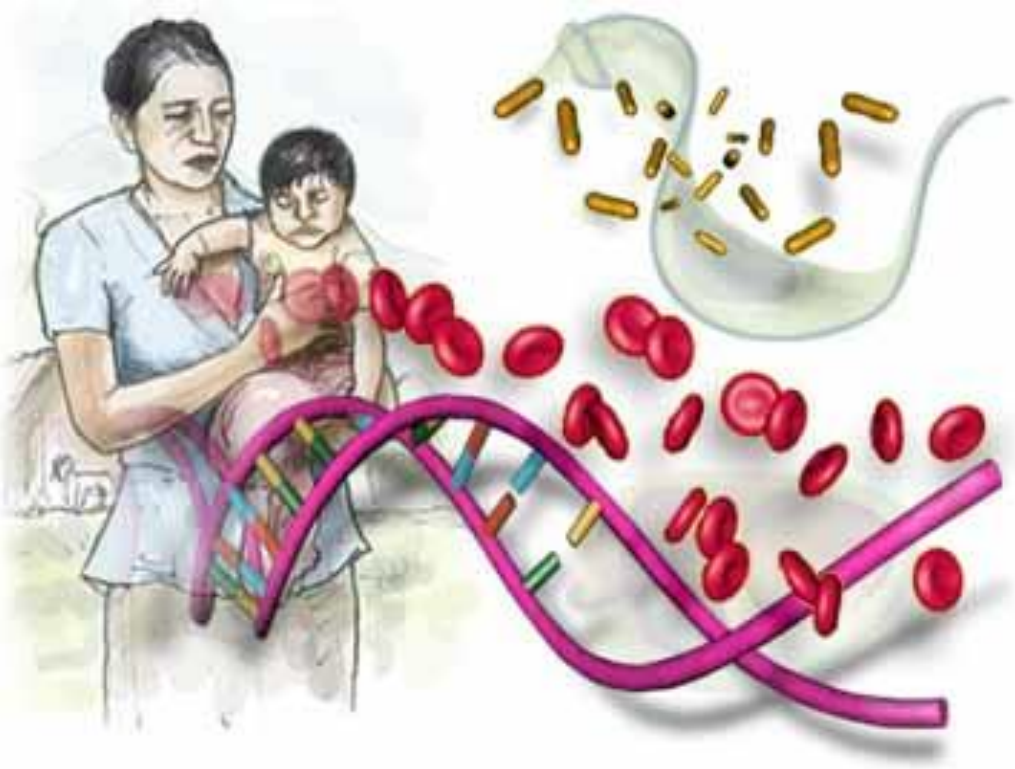
- ***Blood Transfusions and Organ Transplants***

Blood transfusions are the second most common mechanism of transmission of Chagas' disease to people in Latin America, Europe,and the United States.

Transmission methods of T. cruzi

- ***Transmission Through Birth***

Mothers pass *T. cruzi* on to their children as *T. cruzi* travels through the placenta, birth canal, and possibly maternal milk. This type of transmission occurs less frequently than other methods.



Possibilites include diffusion of the parasite across the extra-embryonic membranes, or through the maternal blood supply.

Geographic

- Chagas disease is found only in Latin America

- Natural foci of Chagas disease exist among wild mammals and their associated triatomines.
- Humans and domestic animals became involved in the epidemiologic chain several centuries ago, when insects living under wild conditions began adapting to households.
- Opossums, armadillos, and wild rodents are reservoirs of the parasite, linking the wild and domestic cycles

Clinical Symptoms.

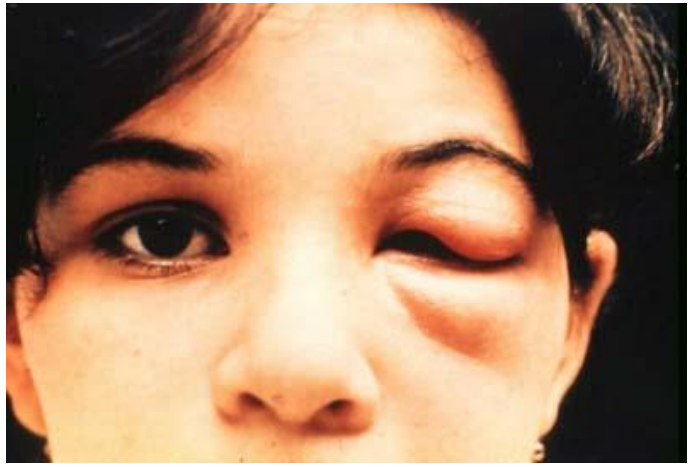
The incubation period is 7-14 days.

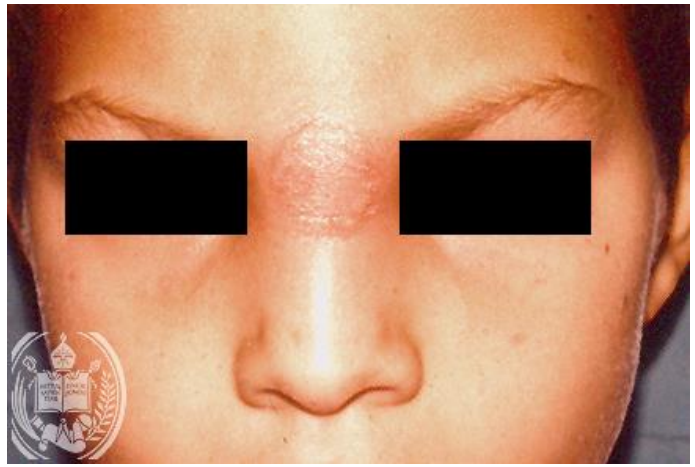
The human disease occurs in 3 stages:

- the acute stage shortly after the infection;
- the indeterminate stage;
- the chronic stage that may develop over 10 years.

Acute phase of Chagas disease

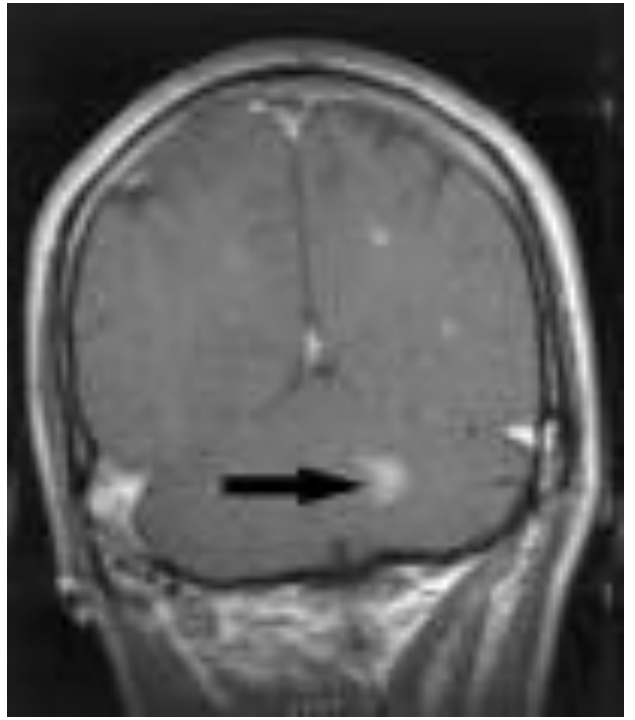
- A local skin nodule called a chagoma can appear at the site of inoculation.
- When the inoculation site is the conjunctival mucous membranes, the patient may develop unilateral periorbital edema, conjunctivitis, and preauricular lymphadenitis. (Romaña's sign).
- The acute phase is usually asymptomatic, but may present symptoms of fever, anorexia, lymphadenopathy, mild hepatosplenomegaly, and myocarditis.





Other symptoms are:

- tiredness,
- sometimes a rash,
- loss of appetite, diarrhea, and vomiting.
- Infants and very young children can get an often-fatal swelling of the brain.



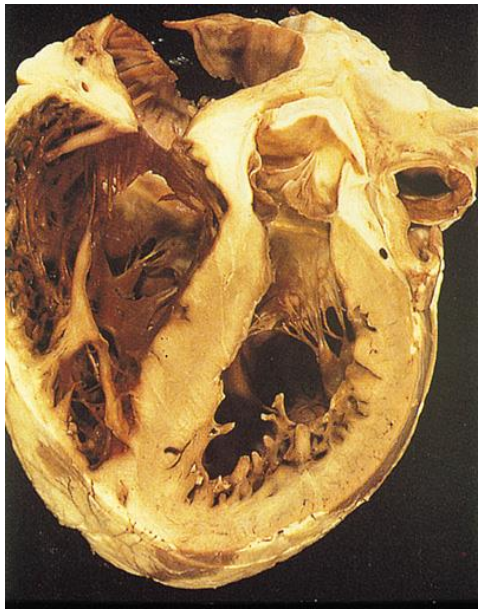
Indeterminate stage

- During the indeterminate stage, about 8 to 10 weeks after infection, infected persons have no symptoms.

Chronic stage of Chagas disease

The disease affects the nervous system, digestive system and heart:

- dementia,
- damage to the heart muscle (cardiomyopathy), altered heart rate or rhythm,
- sometimes dilation of the digestive tract (megacolon and megaesophagus),
- Weight loss.
- Swallowing difficulties may be the first symptom of digestive disturbances and may lead to malnutrition.
- Left untreated, Chagas disease can be fatal, in most cases due to the cardiomyopathy component.



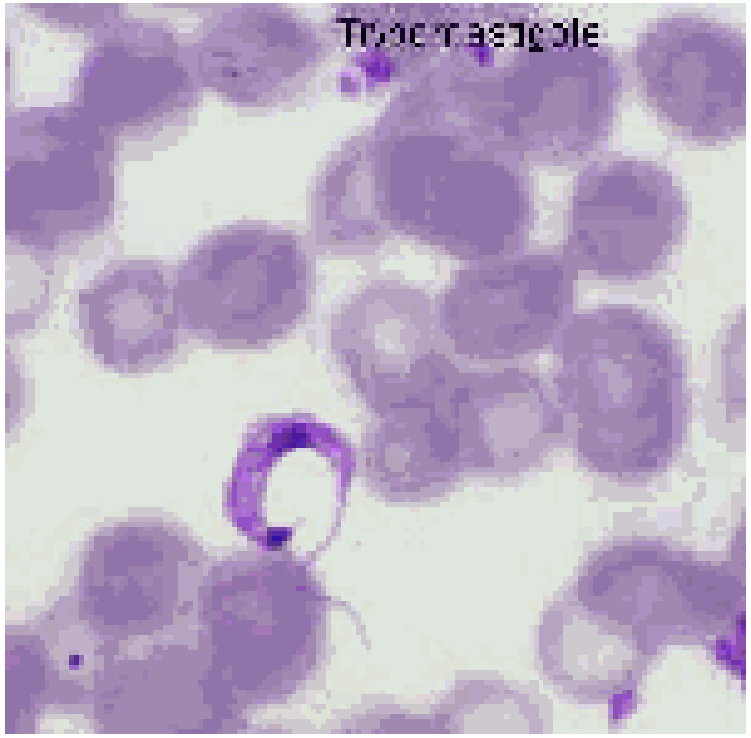
Laboratory Diagnostics

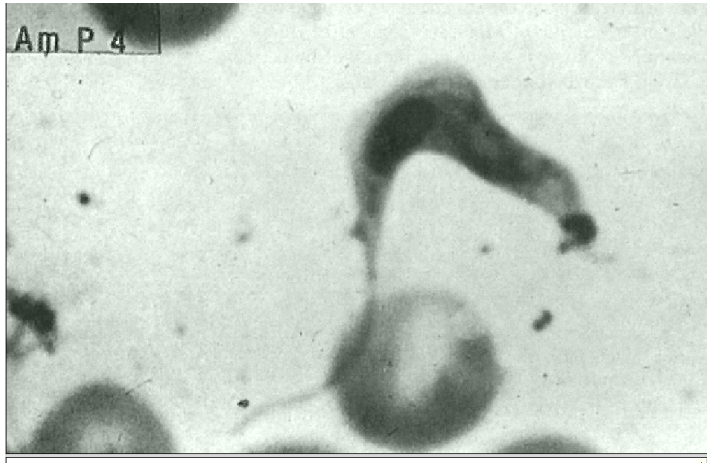
- microscopic blood examination,
- Xenodiagnosis;
- by culturing the blood.
- serologic tests :

➢indirect hemagglutination,

➢indirect immunofluorescence,

➢enzyme-linked immunosorbent assay (ELISA)]

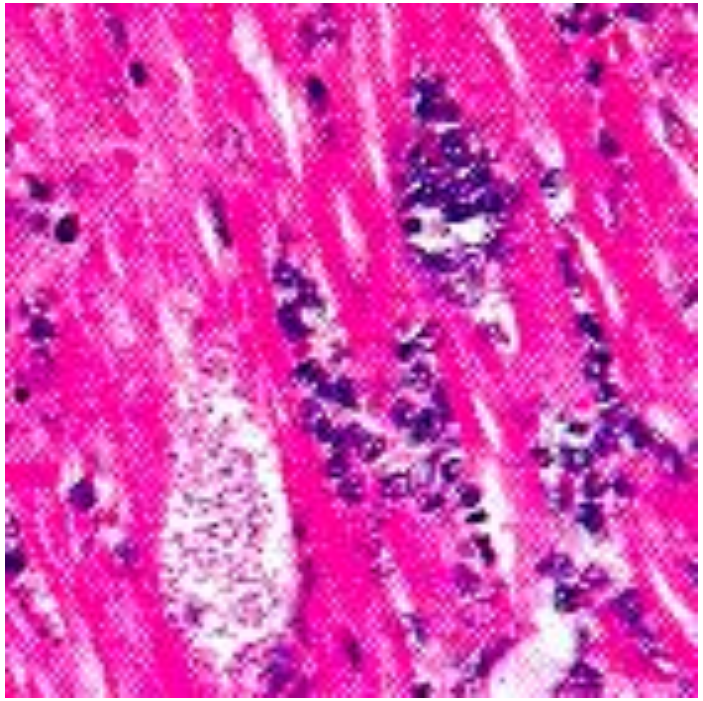




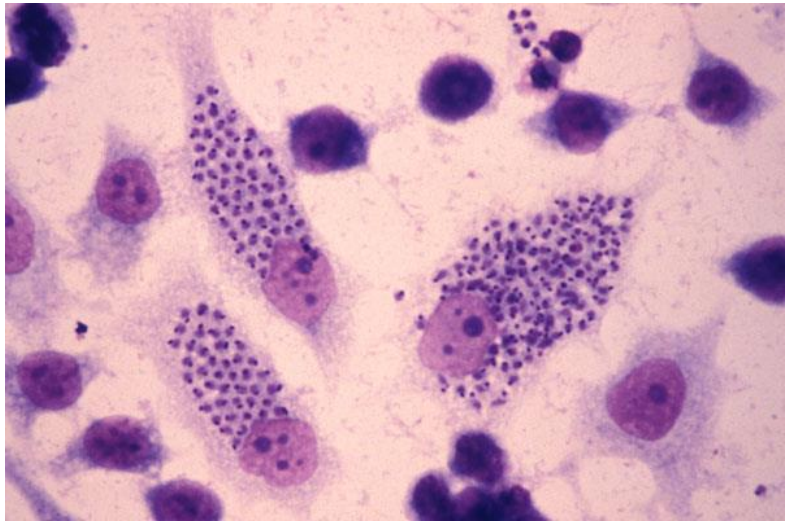
Xenodiagnosis

- In this test, uninfected *vinchucas* are placed in a jar and tucked under the armpit of a patient suspected of being infected.
- The *vinchucas* are allowed to consume blood for thirty minutes, and their feces are examined for *T. cruzi* thirty and sixty days afterward.
- This technique is rarely used on children, and many adults have are hesitant in being willfully bitten by *vinchucas*.

Culturing the blood.



Amastigotes infecting cells of muscle tissue



***Trypanosoma cruzi* in cultured HeLa cells (Giemsa)**

Treatment

- No effective treatment.
- Available drugs only kill extracellular parasites.
- Benznidazole and Nifurtinox: current drugs of choice. Required daily for up to 2 months or more.
- Hospitalization may be needed because of adverse effects

Preventation

- There is no vaccine or drug to prevent Chagas disease. When traveling to areas where Chagas disease occurs, follow these precautions:
- Avoid sleeping in poorly constructed thatch, mud, or adobe houses. If that is not possible, use a bednet.
- Use insecticides to kill insects and reduce the risk of transmission.
- Be aware of the risk of contracting Chagas disease through blood transfusions. In many countries, the blood supply is not well screened.