Lecture VI

The causative agents of dental protozoa and fungi infections

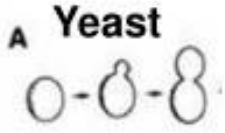
Fungi

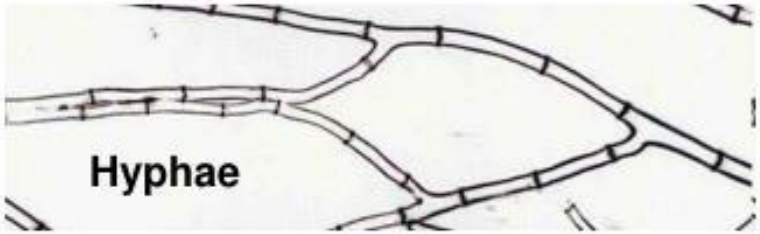
•Of the 50-250,000 fungal species less than 200 cause human disease and only a dozen or so on a regular basis

•Yeasts: unicellular fungi reproduce by budding

• Moulds (filamentous): produce hyphae and mycelium

• Dimorphic: grow as moulds (environment) or yeasts (in human host)





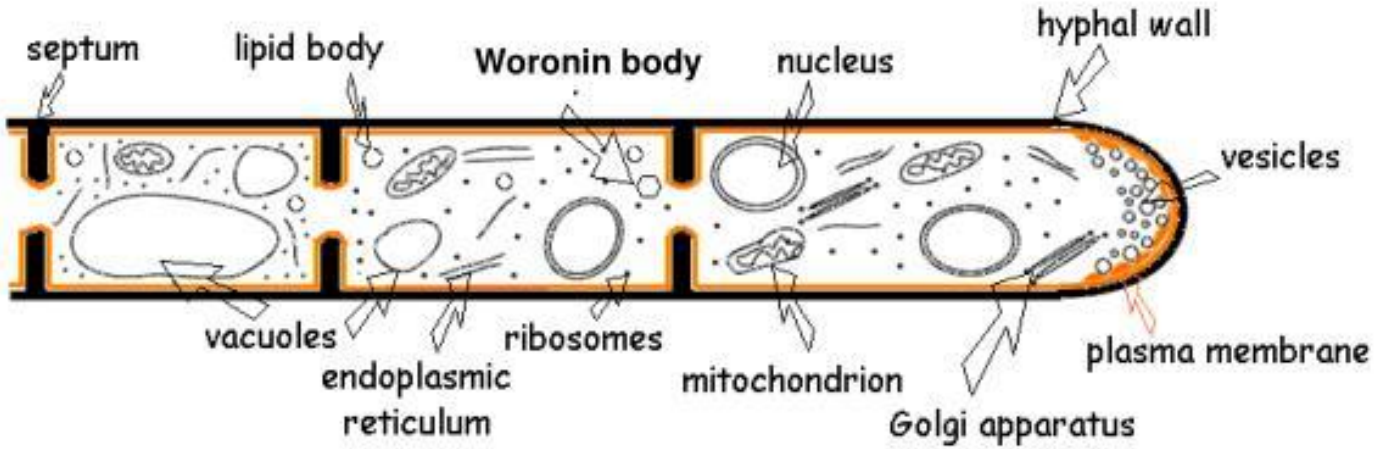
- **Fungi** are **eukaryotic** organisms.
- Their cell wall consists of **chitin**.
- Their cell membrane contains **ergosterol**.

Prokaryotes (Bacteria)	Eukaryotes (Fungi)
0.1-10 um	10-100 um
No nuclear membrane	Nuclear membrane
Single chromosome	multiple
No histones	Histones
Binary fission	Mitotic division
No organelles	Organelles
Peptidoglycan	Chitin
No ergosterol	Ergosterol
70 S ribosomes	80 S ribosomes

Fungal morphology

- Yeast
- Mold
- Dimorphic

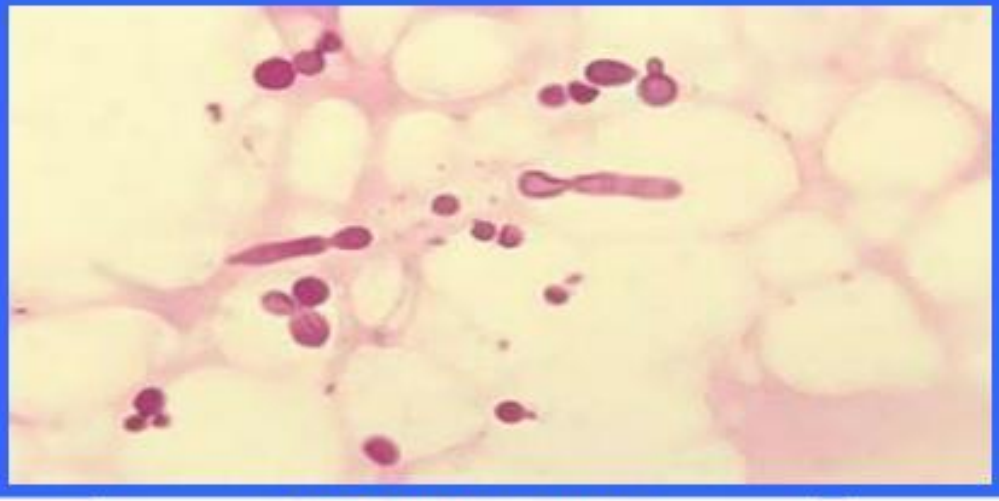
Basic Structure



Hyphal Tip

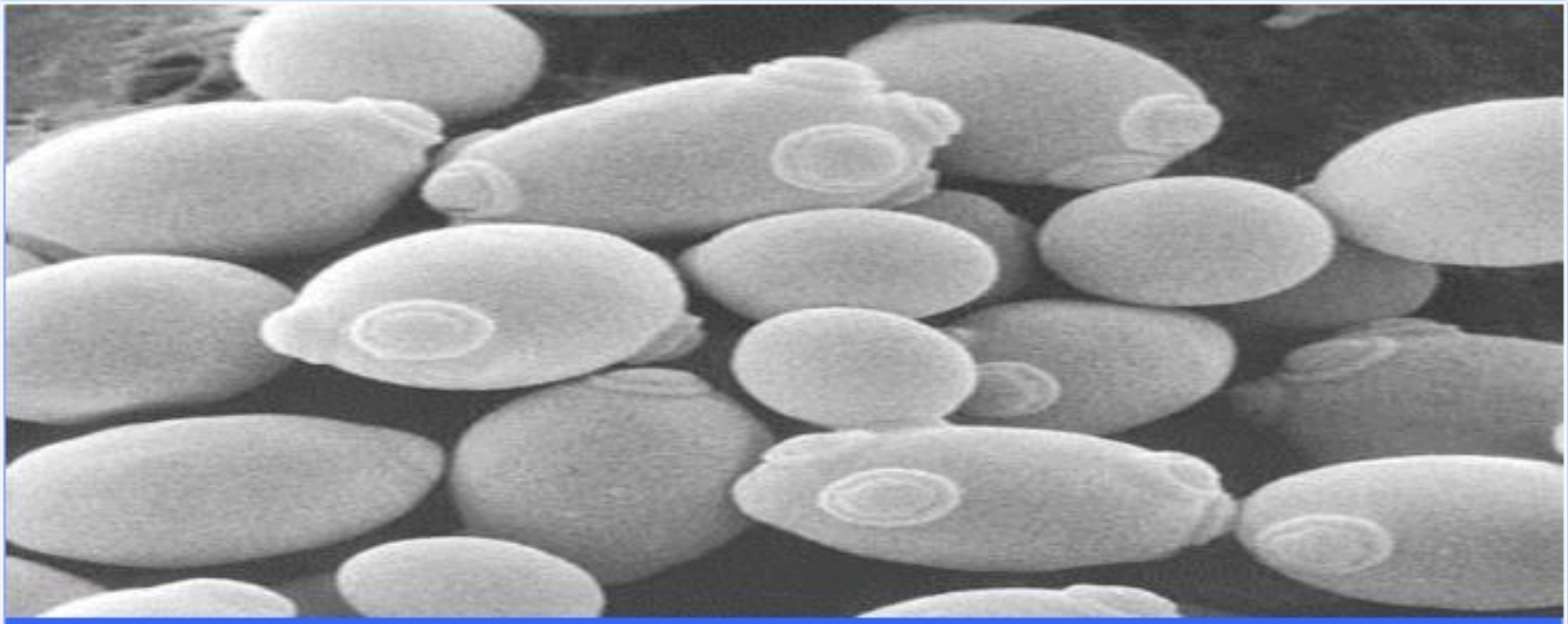
Yeasts

- Oval or round cells that reproduce by budding to form blastospores.
- May form pseudohyphae (if blastospores remain attached to each other).
- Examples: Candida, Cryptococcus.



Molds

- Also called ***filamentous*** fungi or ***mycelial*** fungi.
- Formed of filaments called ***hyphae***.
- Hyphae interlace to form ***mycelium.***
- Hyphae may be septate or ***aseptate.***
- Reproduce by formation of ***conidia***.
- Conidia may be unicellular (***microconidia***) or multicellular (***macroconidia***).
- Examples are: dermatophytes & aspergillus.

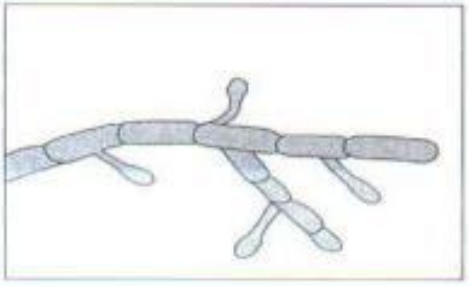


Budding yeast cells

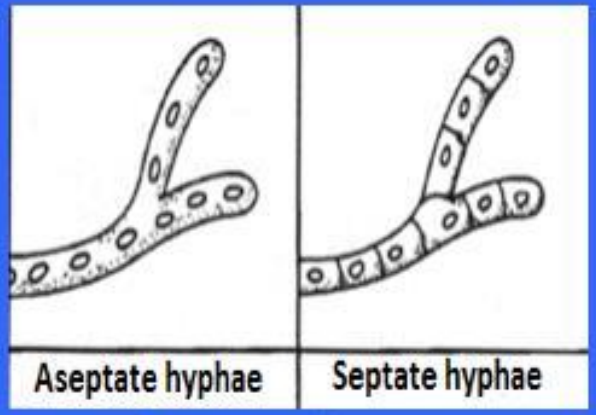
Pseudohyphae

Dimorphic fungi

- These fungi occur in two forms:
  - ❖ At the room temperature (22 degree), it appears as mold.
  - ❖ In the body (37 degree), it appears as yeast cells.
- Examples: Histoplasma & Blastomyces.

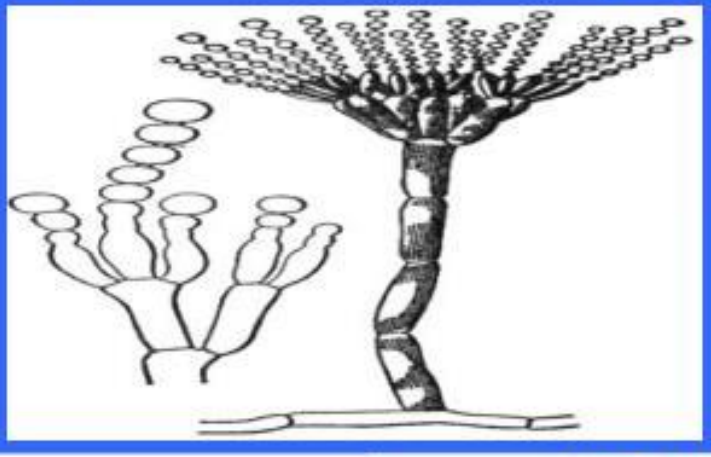
At 22 degree	At 37 degree
	

Hyphae



Mycelium

Microconidia



Macroconidia

Groups of Fungal Infections:

1) Superficial Mycoses
2) Cutaneous Mycoses
3) Subcutaneous Mycoses
4) Systemic Mycoses
5) Opportunistic Mycoses
6) Actinomycetous Infections

SUPERFICIAL MYCOSES

- Pityriasis versicolor
- Tinea nigra
- Black piedra
- White piedra
- Keratomycosis

Superficial Mycoses

- These affect the uppermost dead layers of skin or hair shaft.
- They are painless and usually do not provoke the immune system
- They primarily include:

1- Tinea versicolor (= Pityriasis versicolor)

Brown or discolored or white patches on the skin.

2- Tinea nigra (T.n. palmaris)

Dark brown or grey macular lesions – usually on palm of hand but can be on sole of foot or others.

3- Piedra

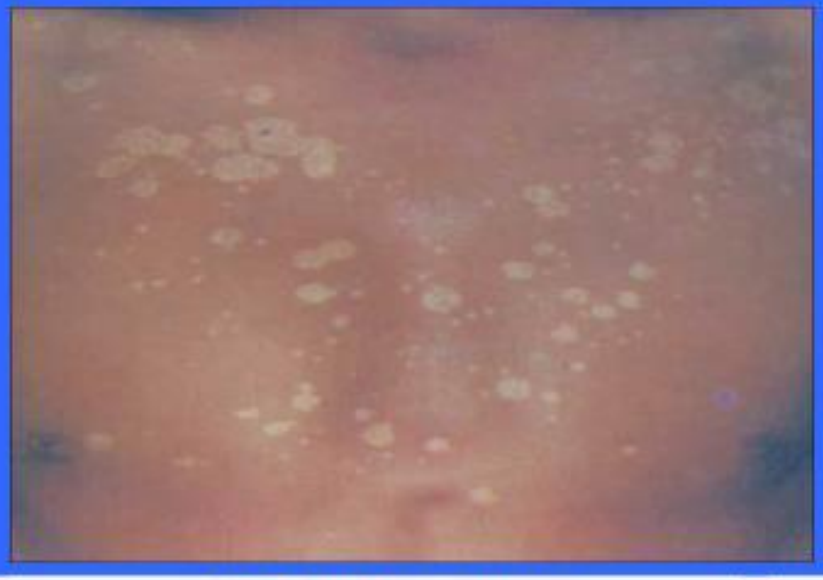
Nodules of the etiologic fungus on hair shaft;

a. Black piedra

b. White piedra

Superficial mycoses

- Fungal infections confined to the stratum corneum without tissue invasion.
- Example: Tinea versicolor caused by Malassezia furfur.



DERMATOPHYTOSIS
Clinical Classification & Manifestations

- Infection is named according to the anatomic location involved:
  - Tinea corporis - small lesions occurring anywhere on the body

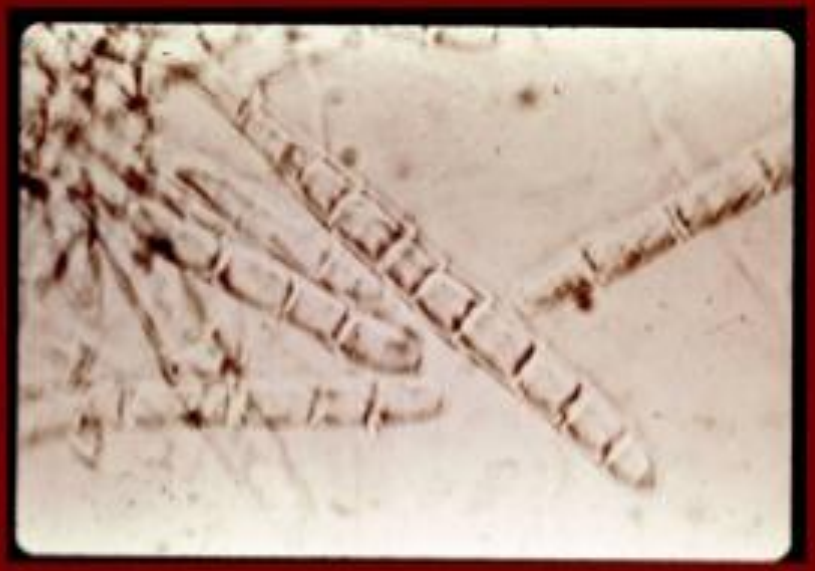
- Tinea pedis - "athlete's foot". Infection of toe webs and soles of feet.

- Tinea unguium (onychomycosis) - nails. Clipped and used for culture

- Tine

- Tinea barbae - ringworm of the bearded areas of the face and neck.

Trichophyton species



Large, smooth, thin wall, septate, pencil-shaped

Trichophyton rubrum

Causes a chronic infection in patients with a cell-mediated immune defect.(Tinea Capitis).

Superficial Mycoses (Continued) AL-HE

Pityriasis Versicolor (= Tinea versicolor)

Brown or discolored, or white patches on skin

Affect the stratum corneum

The white lesions do not tan in the sun

Endogenous source of infection

Etiology: *Malassezia furfur*

It is a Yeast (= Pityrosporum orbiculare)

Blastomycetidae, bipolar budding, Skin flora

Lipophilic: oleic acid Or Mineral oil

Laboratory Diagnosis:

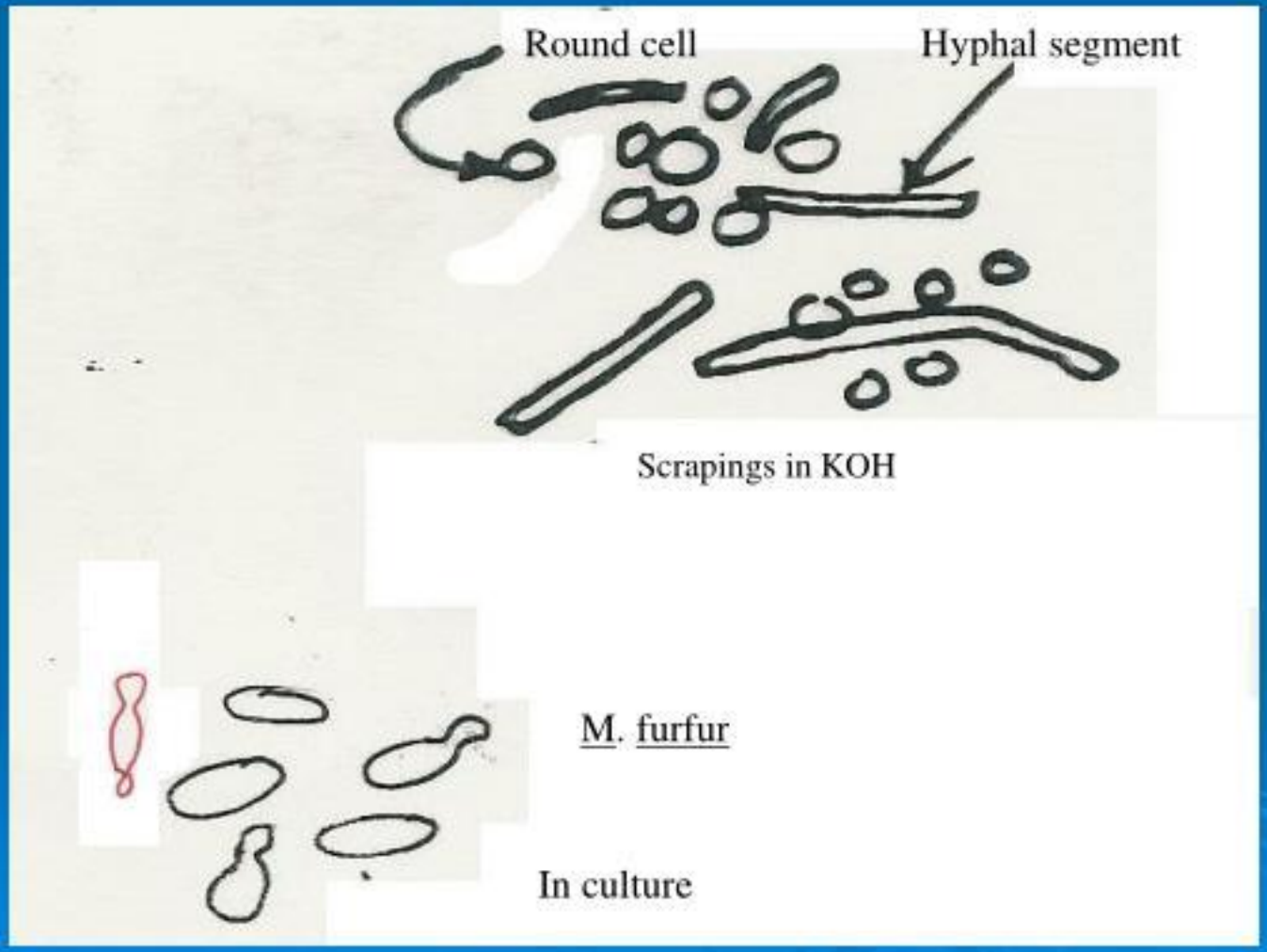
Specimen is skin scrapings

10% Or 20% KOH will show short hyphal segments and

round yeast cells (spaghetti & meat ball appearance)

Superficial Mycoses (Continued)

Pityriasis Versicolor



Subcutaneous mycoses

- Fungal infections that are confined to subcutaneous tissues without dissemination to distant sites.
- Example: mycetoma (madura foot).

Mycetoma (=Madura Foot)

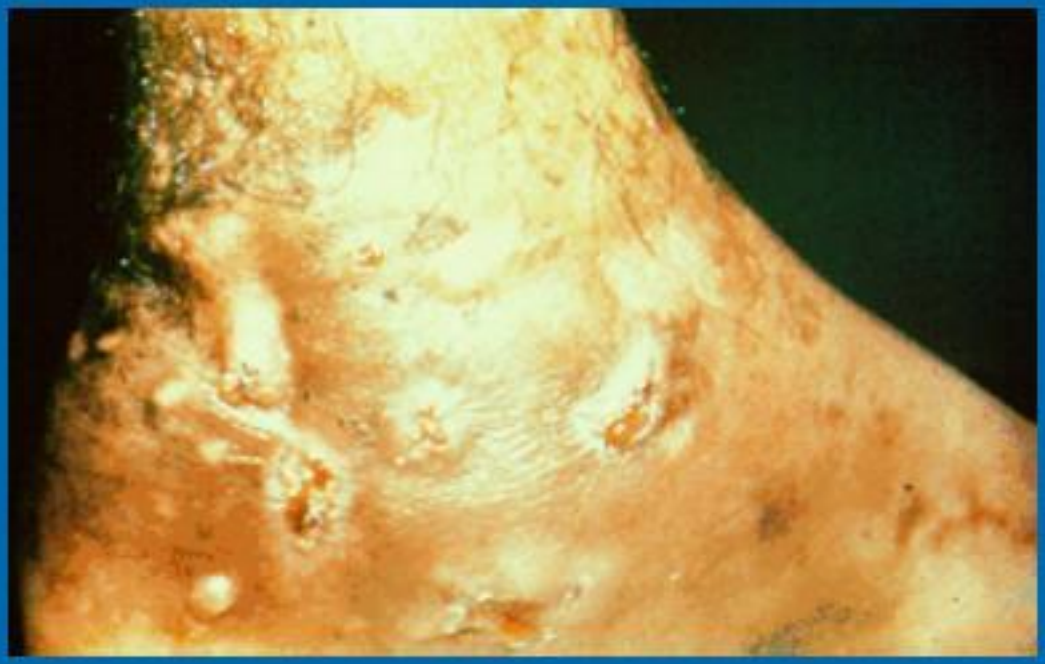
- **Chronic localized subcutaneous infection that involve underlying bone later in the disease course.**
  - The lesions are multiple abscesses.
  - Main symptoms/signes are cold swelling of the affected site (tumefaction), formation of sinuses that drain pus to the surface of the skin, and presence of grains.
  - Grains are granules (small colonies), about 1-2 mm diameter, of the etiologic agent with different color.
  - The commonly affected site is the foot, however, it can be in leg, thigh, hand, arm, shoulder, or head.

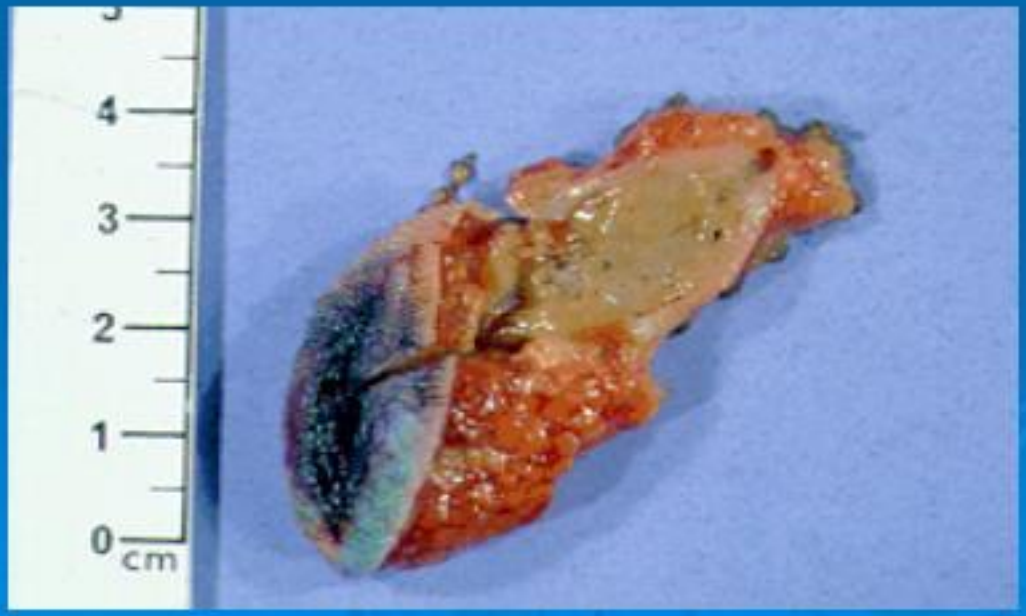
Mycetoma

- Is most common in Africa and South America
- Is a chronic destructive disease affecting skin, underlying tissue and sometimes adjacent bone
- Caused by various fungi including *Madurella* spp., *Scedosporium* spp., *Leptosphaeria* spp.
- Infection results from traumatic implantation of spores into the skin, e.g. thorns, splinters

• **Legend:** Multiple draining sinuses, swollen tissue, and sclerotia are present.

Genus/Species: *Madurella mycetomatis*
Image Type: Clinical Presentation




4
3
2
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cm

Cutaneous mycoses

- Fungal infections that involve keratinized tissues as skin, hair, nail.
- Example: Tinea caused by dermatophytes.

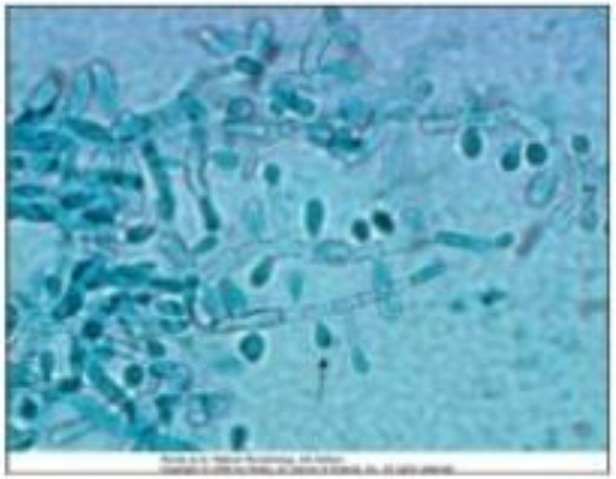
Trichophyton

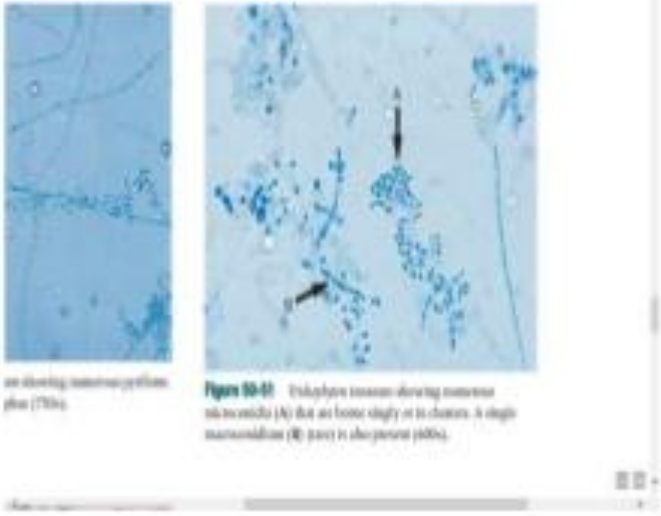
- produces bote micro/macroconidia
- Few or no macroconidia
  - Thin ans smooth
  - fusiform or cylindrical with 2-12 cells per conidium
  - Elongated, few or absent

This micrograph reveals both a macroconidium and some microconidia of the fungus Trichophyton rubrum var. rodhaini.

Trichophyton

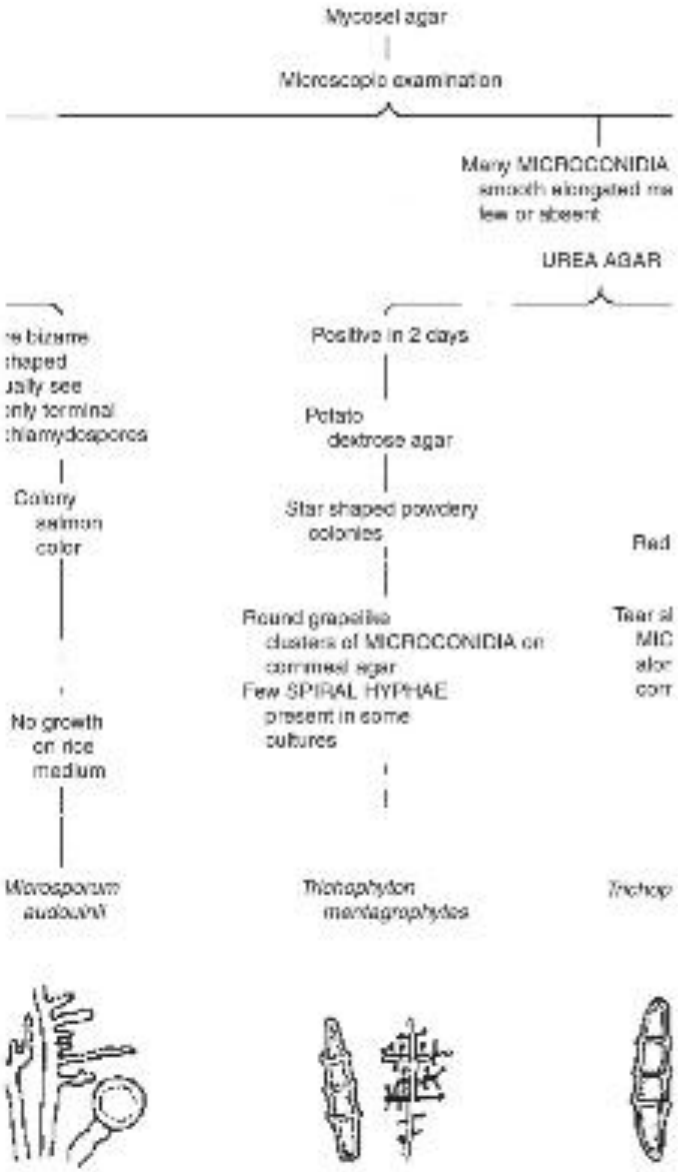
- Many Microconidia
  - Globose, pyriform or clavate
  - Borne on 2 patterns
    - En thryses: sleevelike arrangement around the hyphae
    - En grappe: in clusters (Branches of grapes)
  - Predominant than Macroconidia

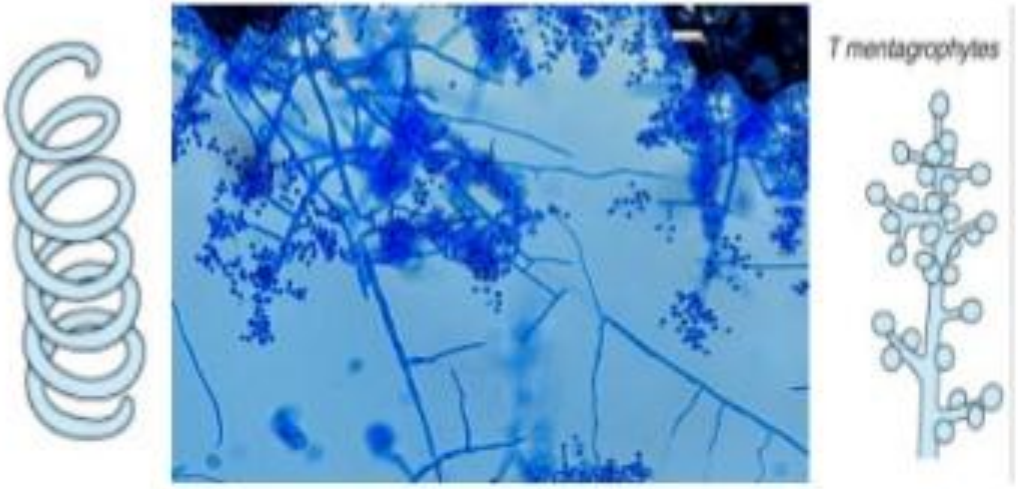




T. mentagrophytes morphology

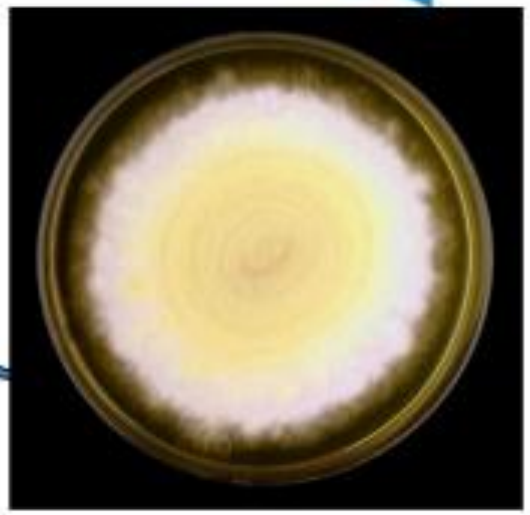
- **Microconidia**
  - **globose but may appear tear-shaped**
  - **are found primarily in grape like clusters, numerous, spiral nodular bodies**
  - **when it is abundant, it will produce granular colonies (Corn meal agar)**





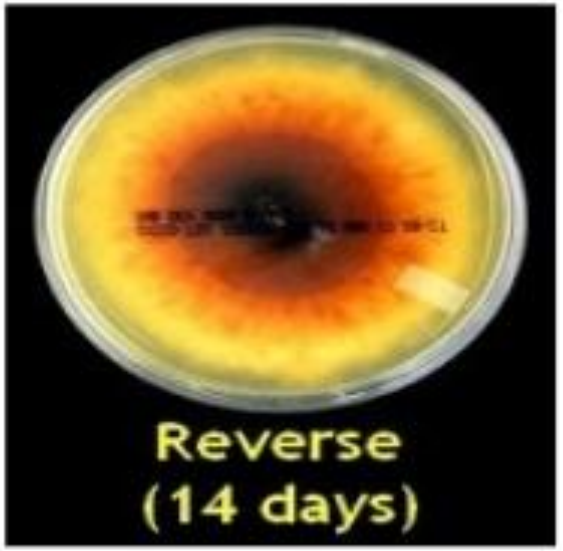
Produces two distinct colonial forms:

- the downy variety recovered from patients with tinea pedis



- and the granular variety recovered from lesions acquired by contact with animals.

- Rose brown on reverse side of colony



Trichophyton tonsurans

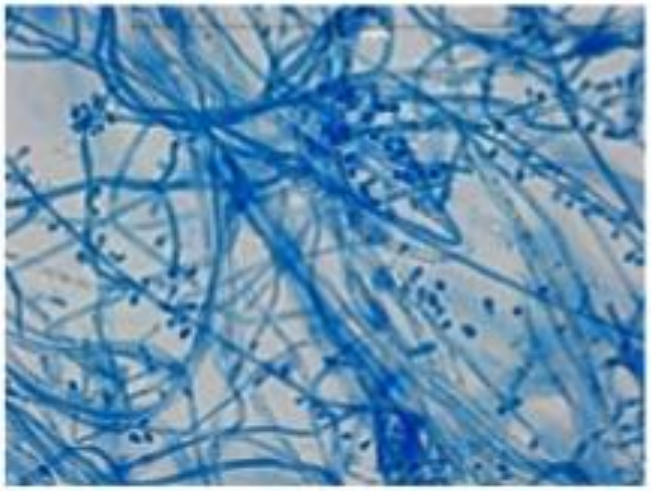
- causative agent of Tinea capitis in children in many parts of the world
- Causes black dot ringworm (hair breaks off)
- Endothrix-hyphae within the hair

- A careful search for the embedded stub should be carried out by the physician with the use of a bright light since it did not fluoresce at Wood's Lamp

• Anthropophilic (prefers humans to animals) however sources vary on its infectivity.

T. rubrum microconidia

- Clavate or peg-shaped, tear shaped along hyphae
- uncommon in most of the fluffy strains but are more common in the granular strains and occur as small,



Trichophyton violaceum infection

- Direct microscopic examination of the calcofluor white or potassium hydroxide preparation of the nonfluorescing hairs shows dark, thick hairs filled with masses of arthroconidia arranged in chains.

Trichophyton shoenleinii

- causes a severe type of infection (tinea capitis) called **favus,** sometimes permanet alopecia- *characterized by the formation of yellowish cup-shaped crusts or scutulae.*
- Organism causes an endothrix-style growth, **but without the arthrocondia.** Instead, channels are formed within the hair shaft.

T. shoenleinii cultural characteristics

- Is a slowly growing organism (30 days or longer) and produces a white to light gray colony that has a waxy surface.
- The reverse side of the colony is usually tan or nonpigmented.
- All strains of T. schoenleinii may be grown in a vitamin-free medium and grow equally well at room temperature or at 35° to 37° C.

Sabouraud dextrose agar, 25°C, 62 days

Potato dextrose agar , 25°C, 27 days.

Epidermophyton floccosum

- Affects Nails and Skin
- Culture: cottony , range of colours
- Microscopy: Bifurcated hyphae with multiple, smooth, club shaped macroconidia (2-4 cells)

- Lab culture and Micros

Systemic mycoses

- Also called endemic mycoses.
- Begin as primary pulmonary lesions that may disseminate to any organ.
- Caused by dimorphic fungi.

Disseminated Coccidioidomycosis

- Fine needle aspirate of the mass revealed spherules filled with endospores

Stafford et. al., Infect Med 24 (Suppl 8): 23-25, 2007.

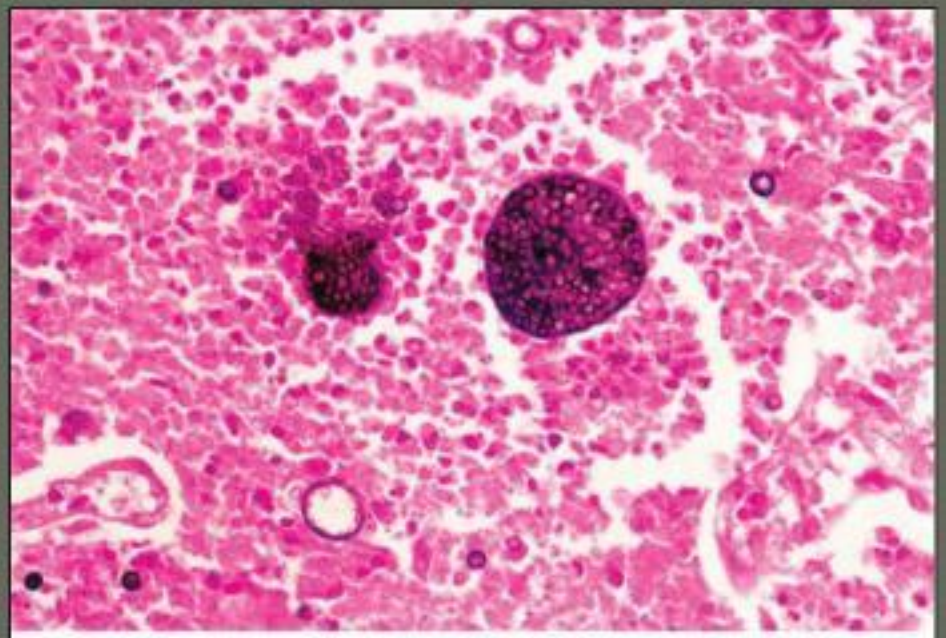
Disseminated Coccidioidomycosis

- Culture grew *Coccidioides immitis*
- Serology panel for *C. immitis* was positive
- CSF = normal
- Bone scan revealed multiple region of increased osteoblastic activity

Stafford et. al., Infect Med 24 (Suppl 8): 23-25, 2007.

Coccidioidomycosis - Manifestations

Coccidioidomycosis



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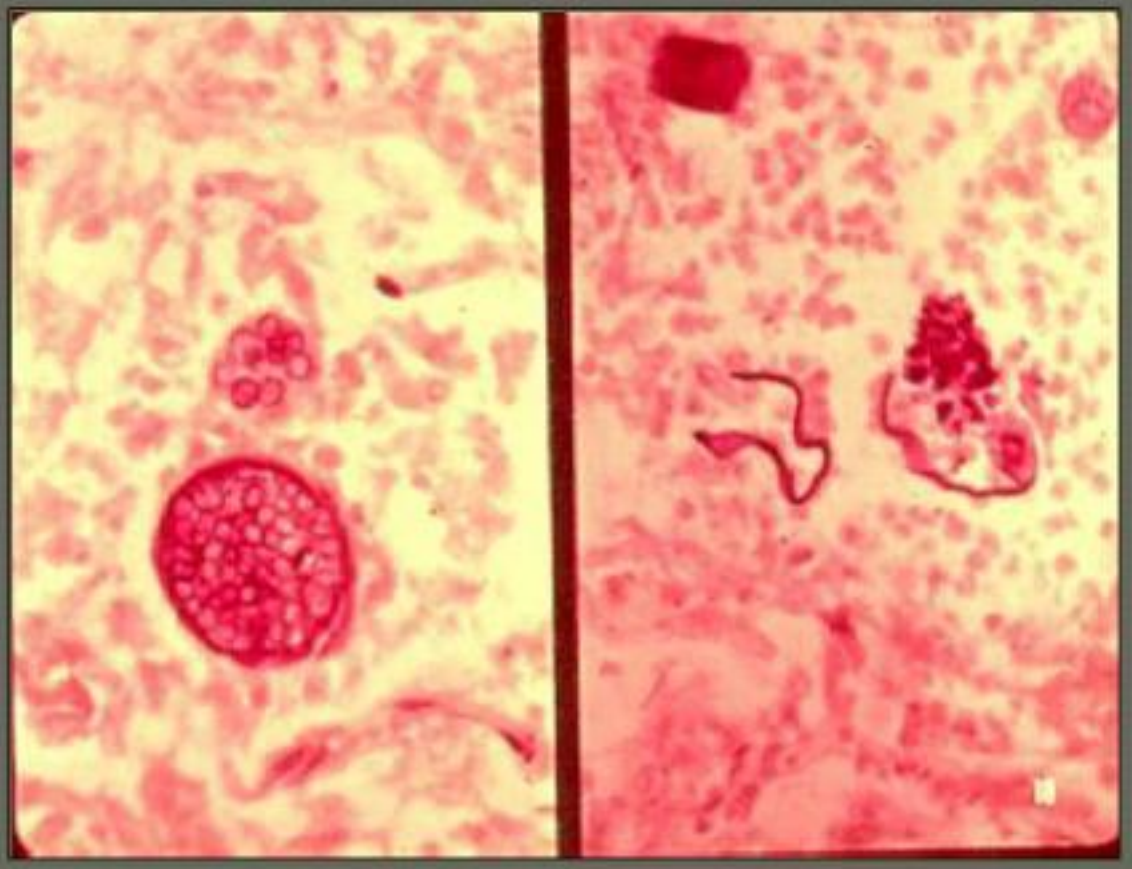
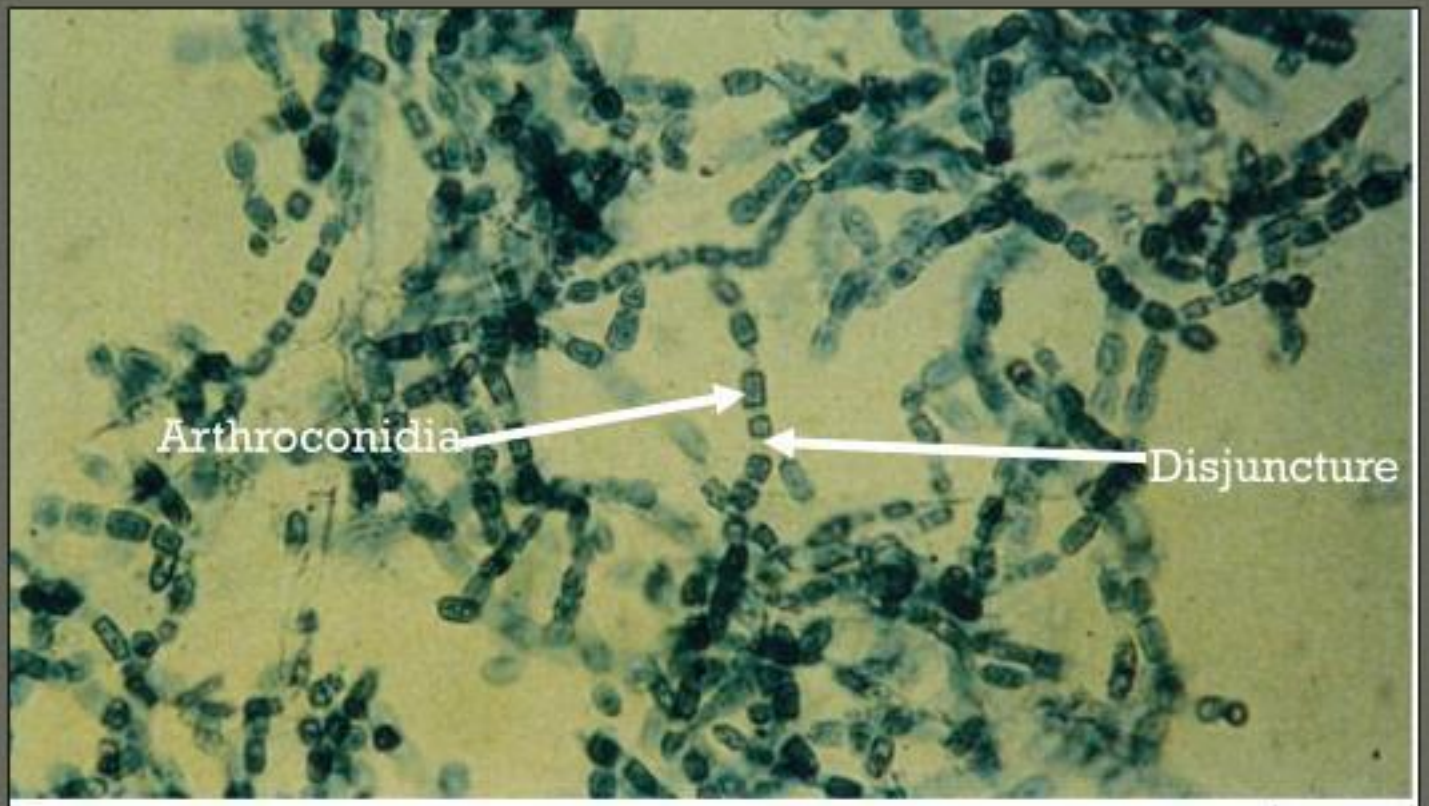
Source: Ryan KJ, Ray CG: *Sherris Medical Microbiology, 5th Edition:* www.accessmedicine.com

Lung tissue with a large thick-walled spherule containing multiple endospores. The smaller spherule to its left has ruptured releasing endospores.

Coccidioidomycosis



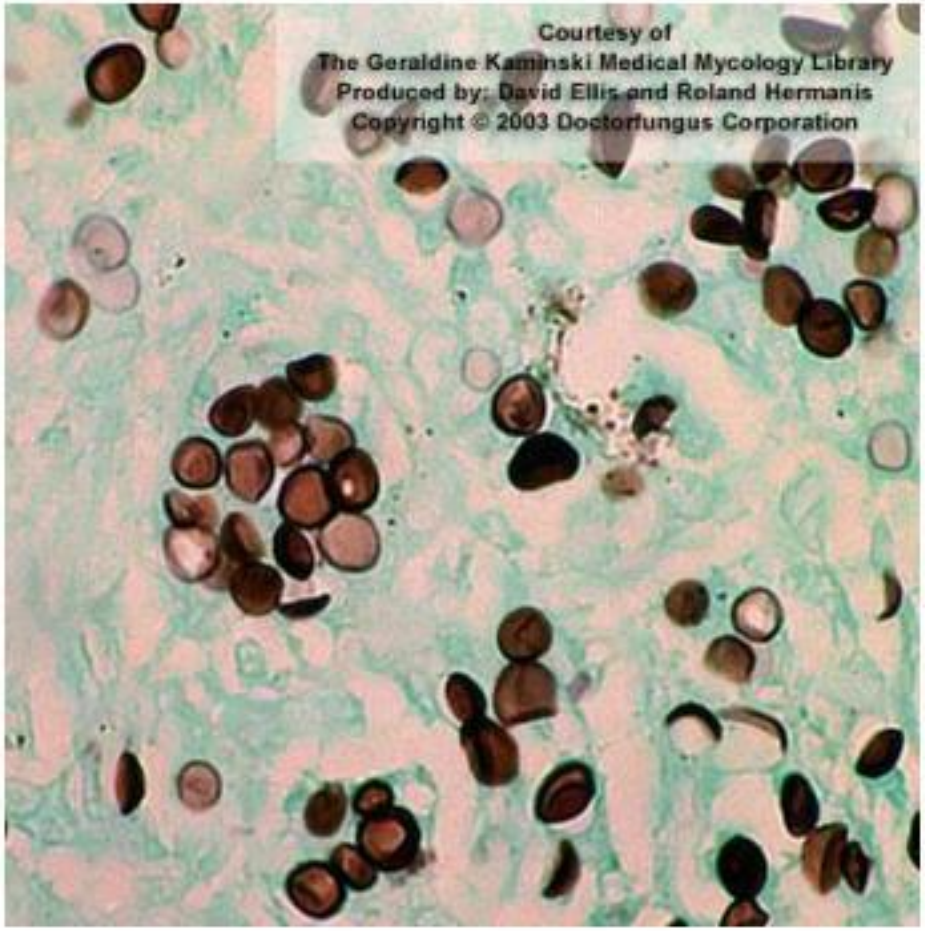
Coccidioidomycosis



Source: Ryan KJ, Ray CG: *Sherris Medical Microbiology, 5th Edition:*
www.accessmedicine.com

Histoplasmosis

- The most common endemic mycosis in North America, also found in Central and South America
- A thermally dimorphic fungus, found as a mould in the environment but as budding yeast in tissue
- Inhalation of spores is the primary route of infection
- Prolonged exposure to aerosolised spores is a major risk factor
- Fewer than 5% of individuals exposed to the fungus develop symptomatic disease

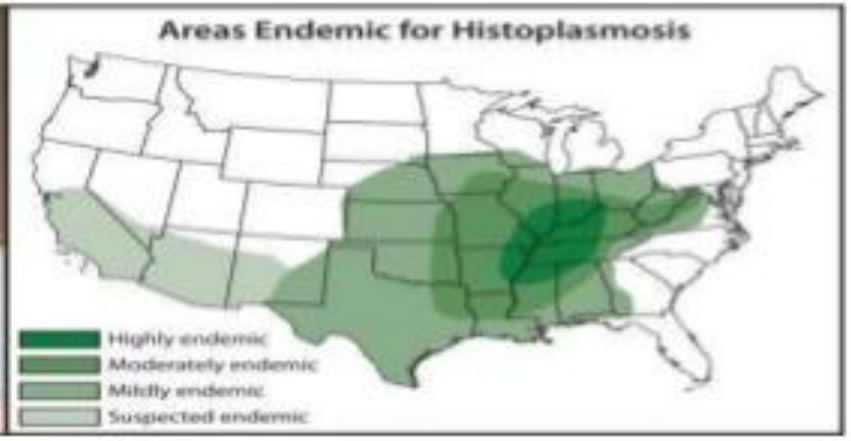


- **Genus/Species:** *Histoplasma capsulatum* var. *duboisii*
- **Image Type:** Histopathology

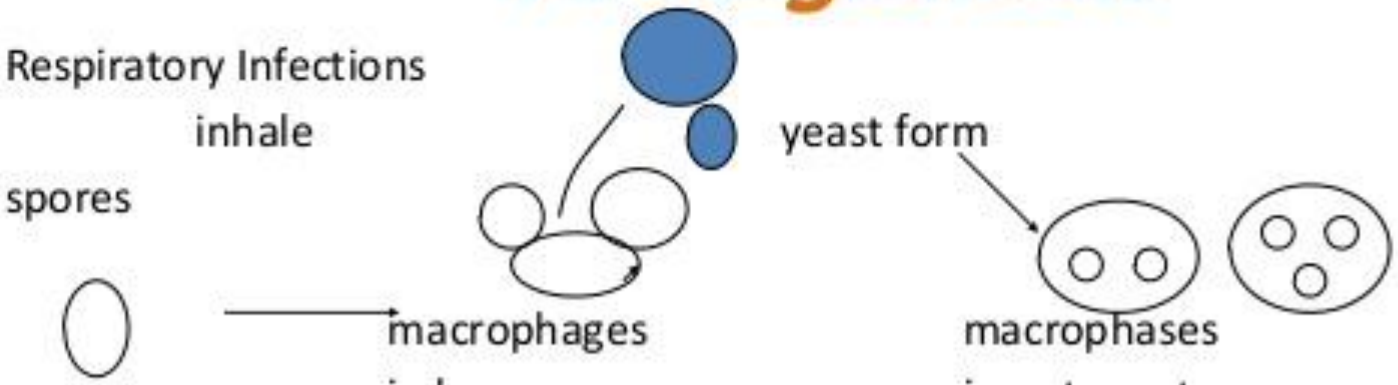
Histoplasmosis

Characteristics

- Member of the phylum Ascomycota
- Worldwide distribution
- Naturally found in fecal-contaminated soils
- Birds and bats appear to be reservoirs
- Etiologic agent of histoplasmosis



HISTOPLASMOSIS
Pathogenesis



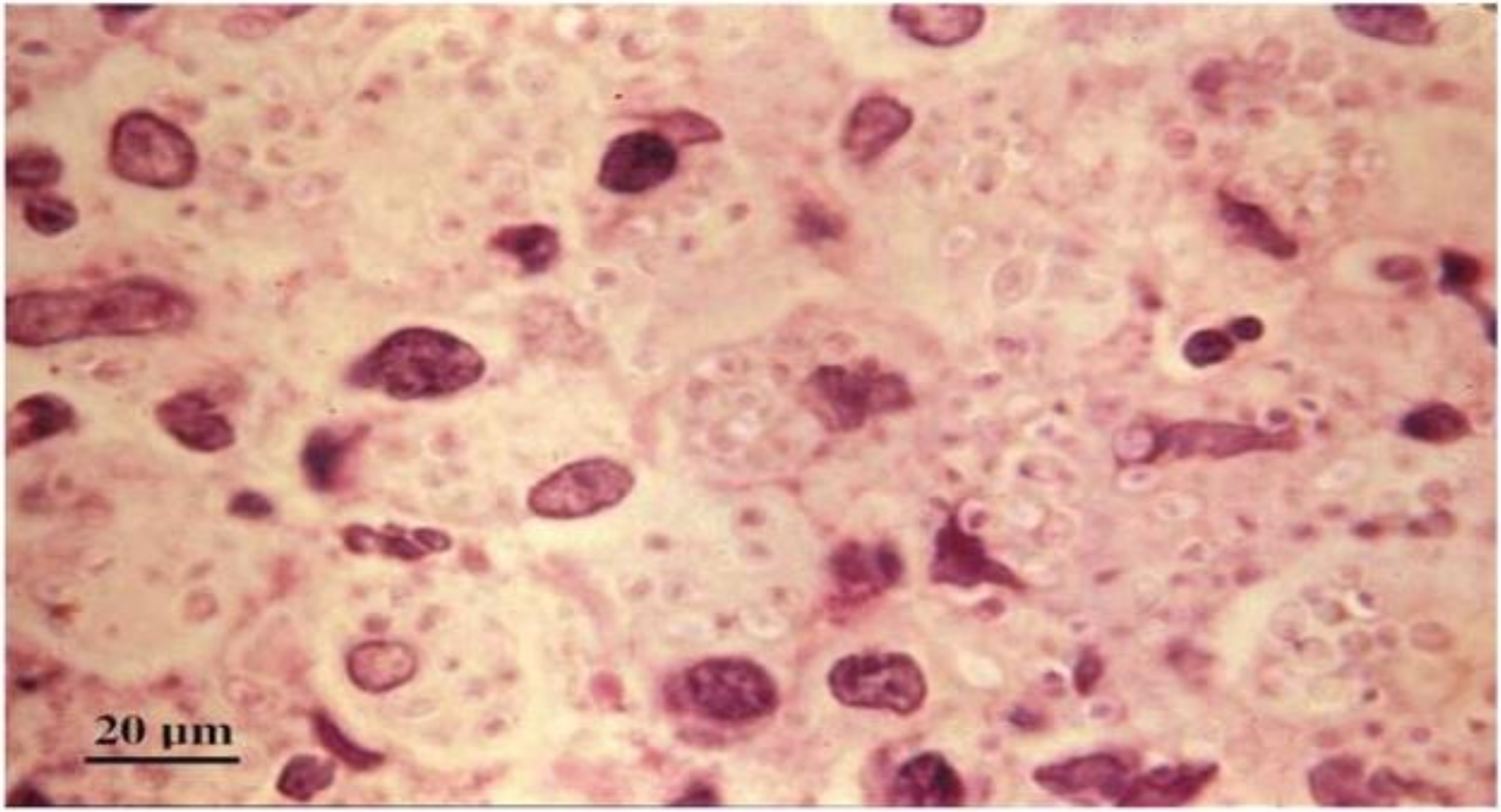
Proliferation halted by onset of acquired CMI at 10-14 d.

Vasculitis, tissue necrosis, **caseating granulomata**. Killing by macrophages, healing, calcification

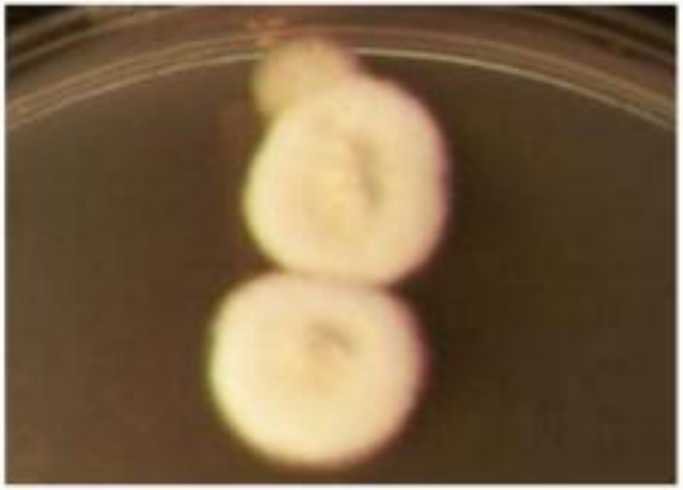
Histoplasmosis

Oral histoplasmosis

Haematoxylin and Eosin (H&E) Stain

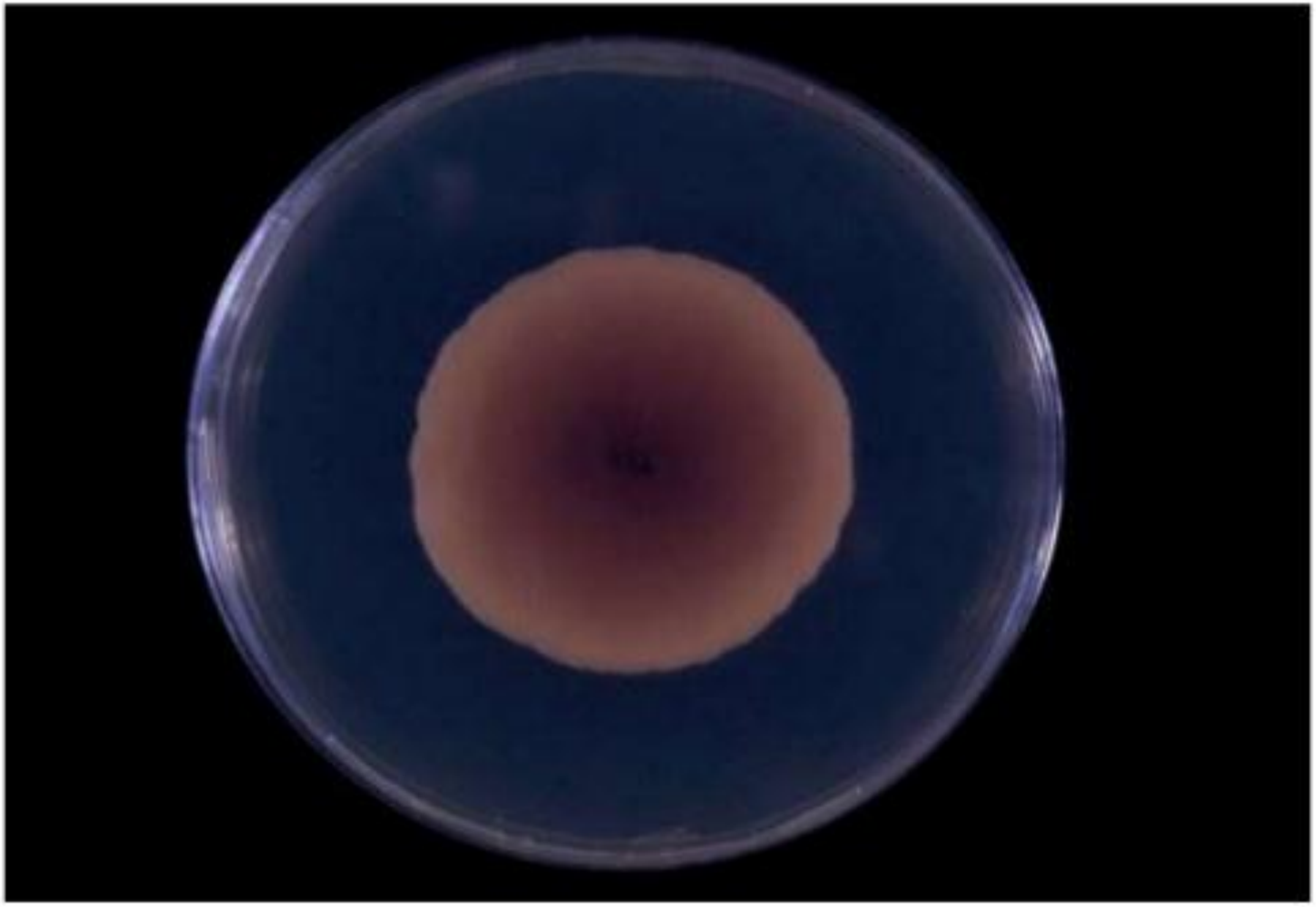


Macroscopic morphology Sabouraud's dextrose agar



Mould at RT

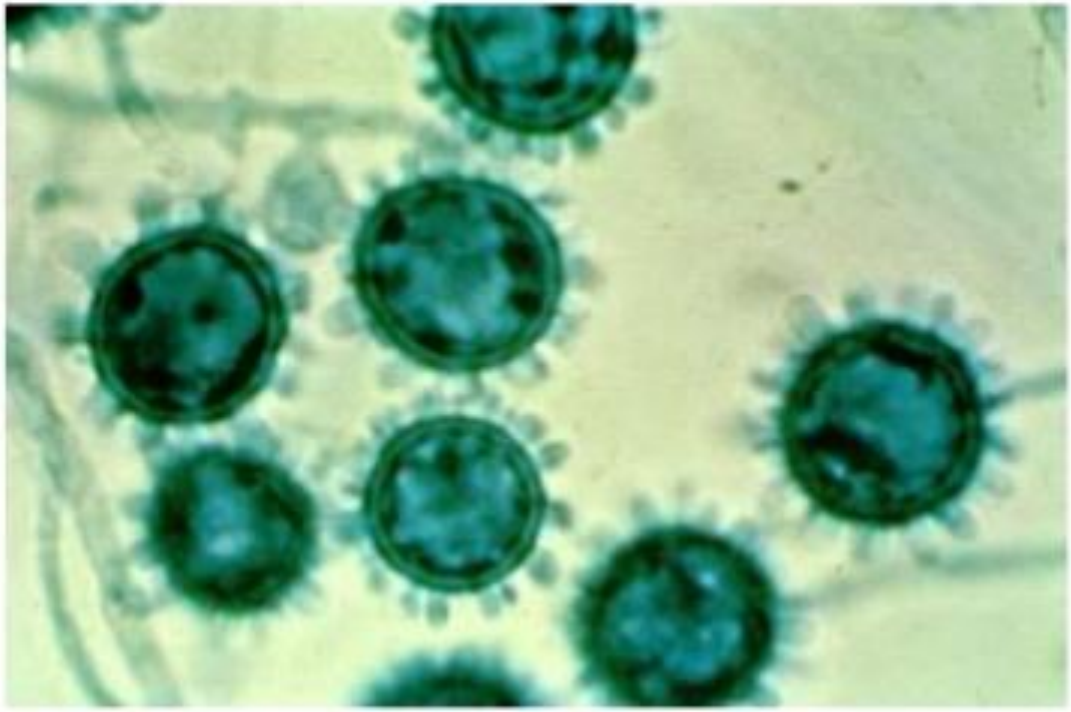
Culture of *Histoplasma capsulatum* on Sabouraud's dextrose agar



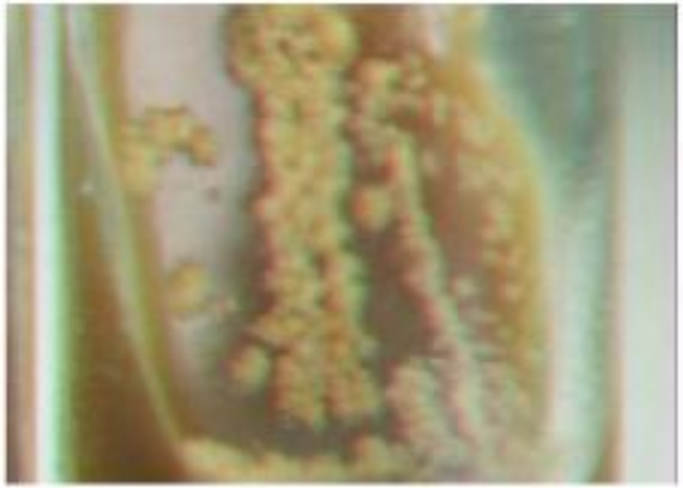
Click icon for audio

Mold - *Histoplasma capsulatum*

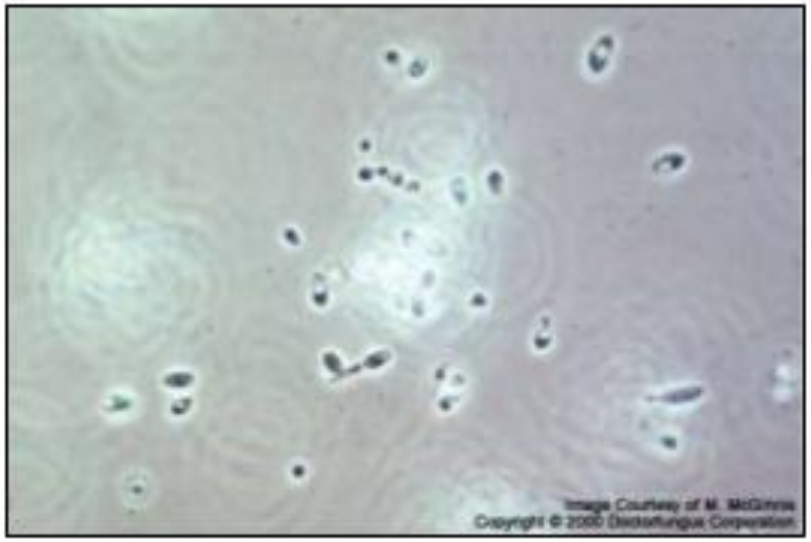
- Microscopic morphology



Hyphal to yeast conversion at 37°C



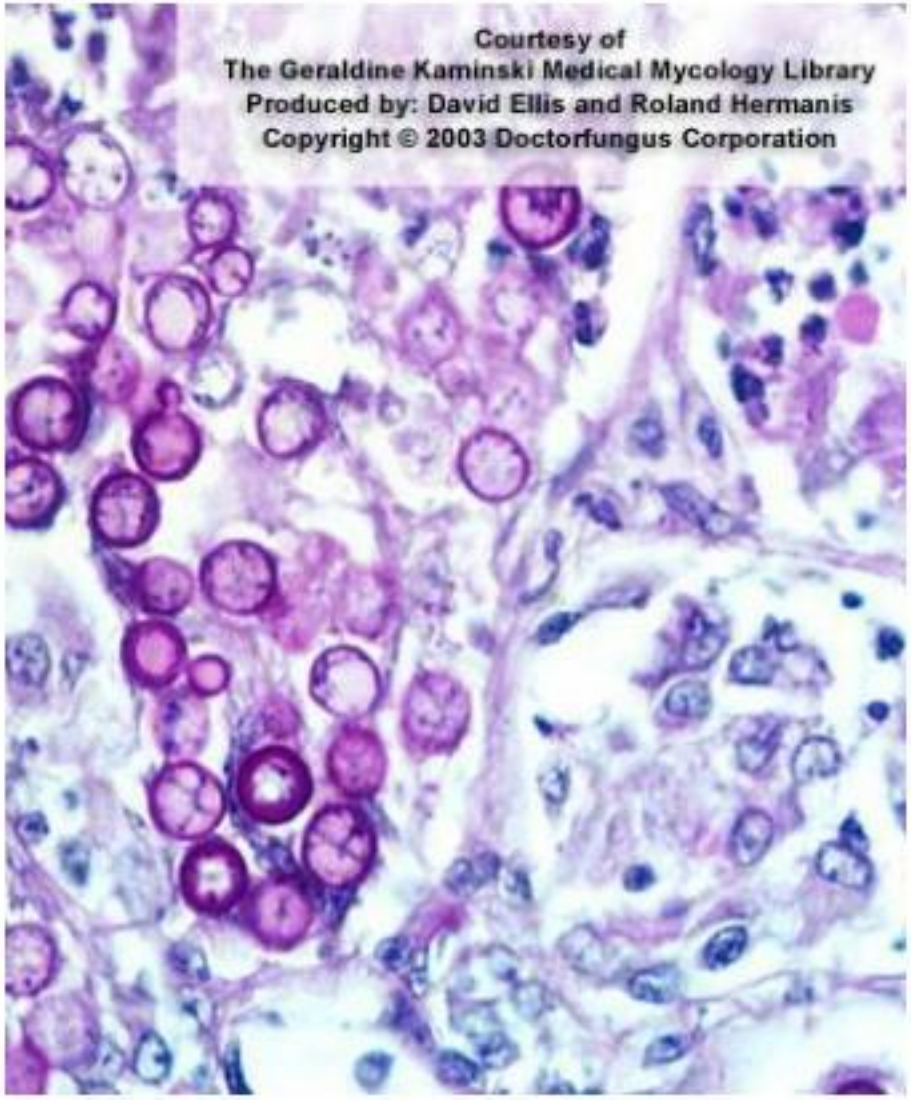
Yeast-like colonies



Yeast cells

Blastomycosis

- Occurs in North and South America, also Africa
- It is a mould in the environment but forms large budding yeast in tissues
- Infection through inhalation
- Normally in individuals with outdoor occupations
- The skin is the most common site of disseminated disease



- **Genus/Species:** *Blastomyces dermatitidis*
  - **Image Type:** Histopathology

Opportunistic mycoses

- **Affect immunocompromised individuals**
- **Examples are:**
  1. ***Candidiasis caused by Candida albicans.***
  2. ***Cryptococcosis caused by Cryptococcus neoformans.***
  3. ***Aspergillosis caused by aspergillus fungus.***
  4. ***Pneumocystis pneumonia caused by pneumocystis jiroveci in AIDS patients.***

Candida spp.

***Candida* Species**

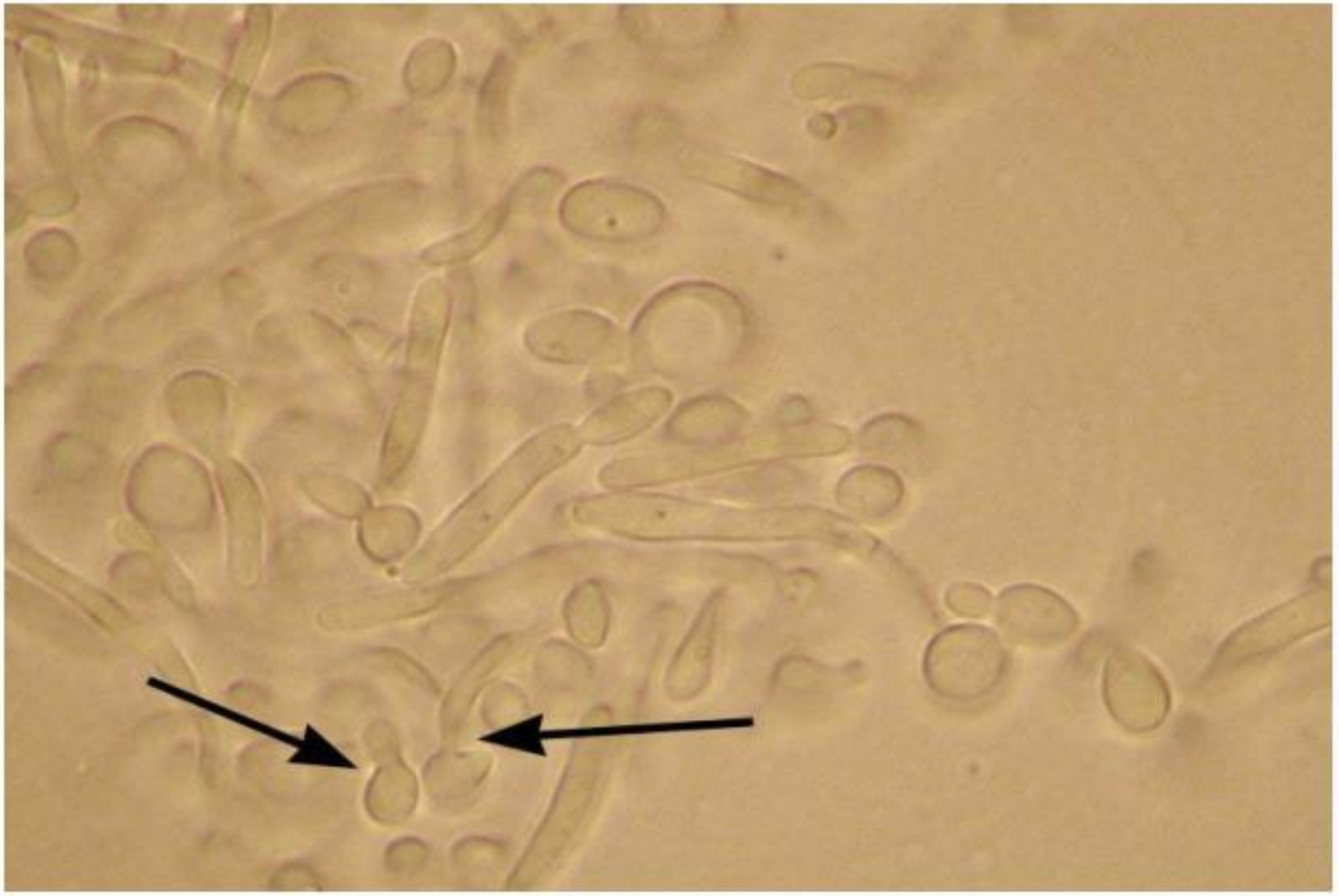
- Generally grow at 37°, ferment glucose and may ferment other carbohydrates, and form pseudo- or true hyphae
- Harbored by the gastrointestinal tract

Candida albicans

- Germ tube positive
- Creamy colonies, as other yeasts
- May display pseudohyphae and true hyphae
- Most commonly isolated candidiasis
- Virulence factors include rapid germination within tissue, protease production, surface integrin-like molecules for binding extracellular matrix, complement protein binding receptor, phenotypic switching, and surface variation and hydrophobicity

Candida albicans, showing pseudohyphae with clamydospores, this fungus usually takes yeast

Candida albicans **on blood agar; moist, opaque colonies are characteristic of yeast.**



Candida albicans incubated in rabbit serum at 37° (germ tube test). Germ tubes are indicated by arrows and are the beginnings of true hyphae: no constriction is at the origin of the germ tube and the parent cell.

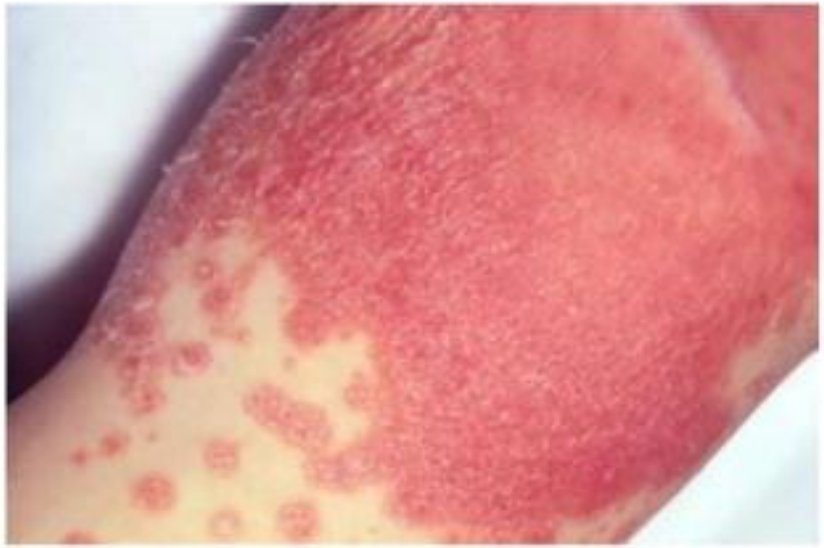
Candidiasis

- **Candidiasis**, caused by *Candida albicans*, or lesser by *C. tropicalis* and *C. glabrata*.
- The species is commonly present in human mucosa, which becomes invasive when immunity weakens.
- In newborns natural resistance is low, candidiasis can develop within a few days.
- Disseminated Cadidiasis can be fatal when untreated.

CHRONIC CANDIDIASIS (onychomycosis) of thumb nails showing destruction of nail tissue.

Superficial candidiasis in an infant (nappy rash)

Interdigital candidiasis of the hands



Intertriginous or flexural candidiasis behind the knee showing an extensive erythematous scaling lesion

ORAL CANDIDIASIS

- also known as **"thrush"** is an infection of yeast fungi of the genus *Candida* on the mucous membranes of the mouth.

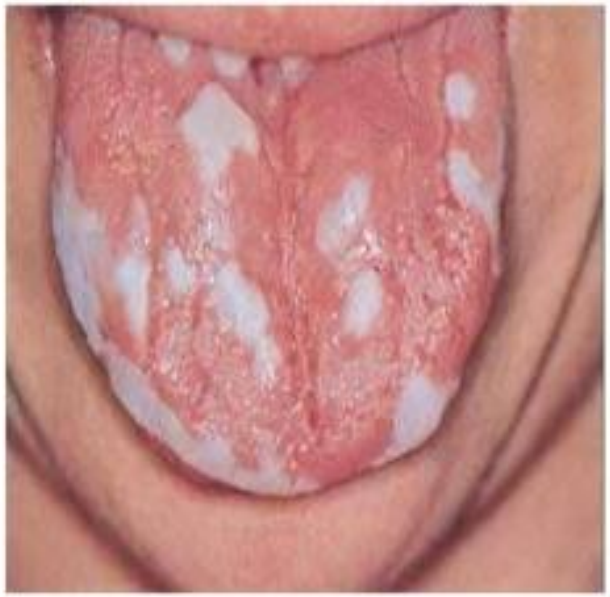
(Candida albicans, *Candida glabrata* **or** *Candida tropicalis*.

- Can occur due to dryness of the mouth or environmental irritants.

- **candidiasis - mouths of babies**
- ***candidosis* or *moniliasis*- mouth and throat of adults**

- The main forms of candidiasis that produce white lesions are the following

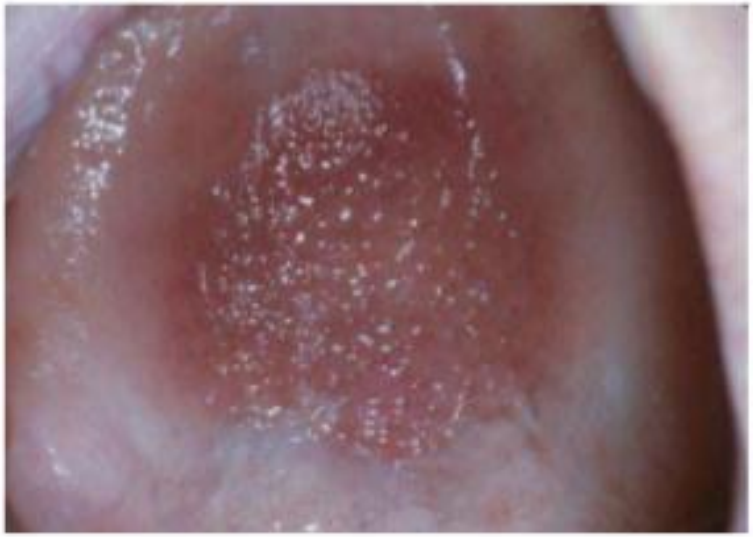
Pseudomembrabous candidiasis



Mucocutaneous candidiasis

Nodular candidiasis

Erythematous



Papillary Hyperplasia of the Palate

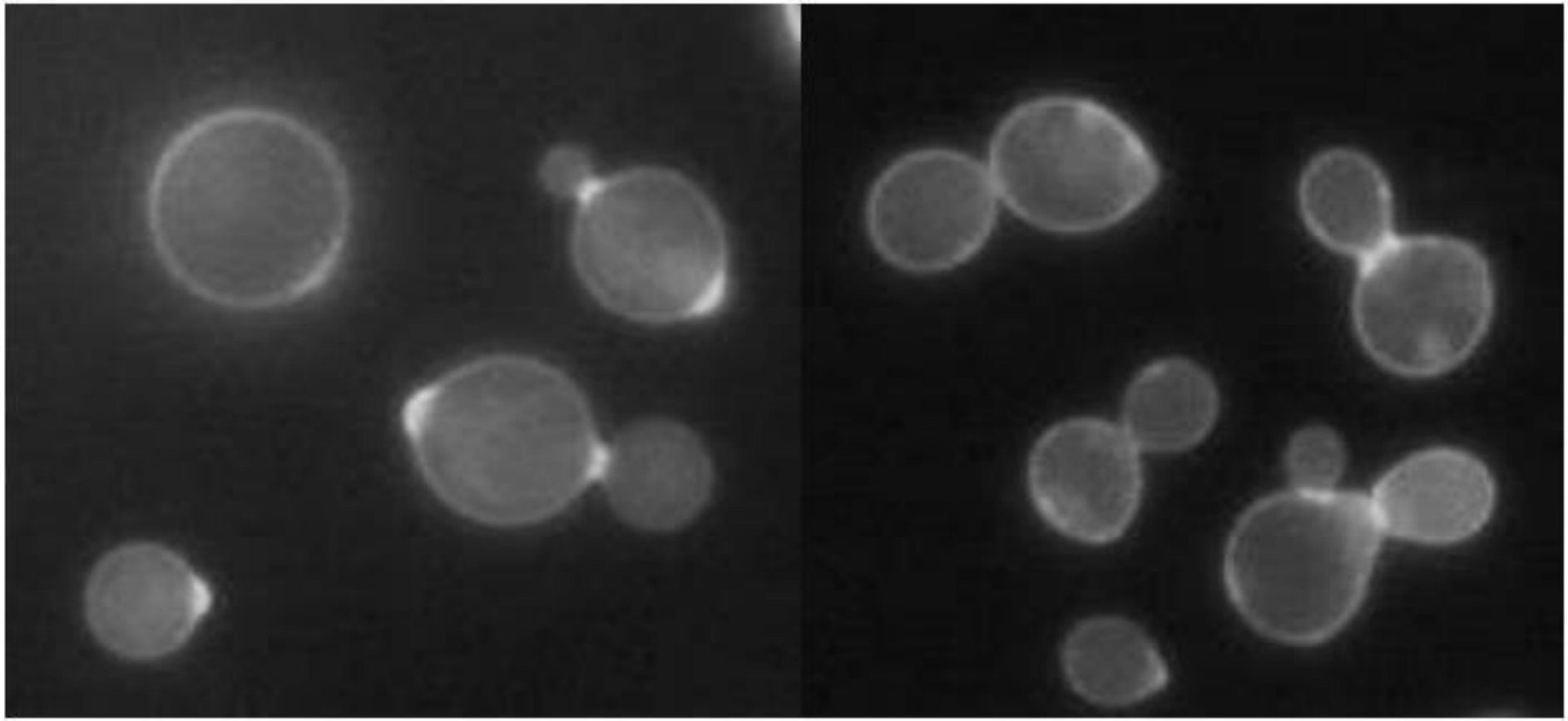
angular cheilitis

Denture-Related Stomatitis

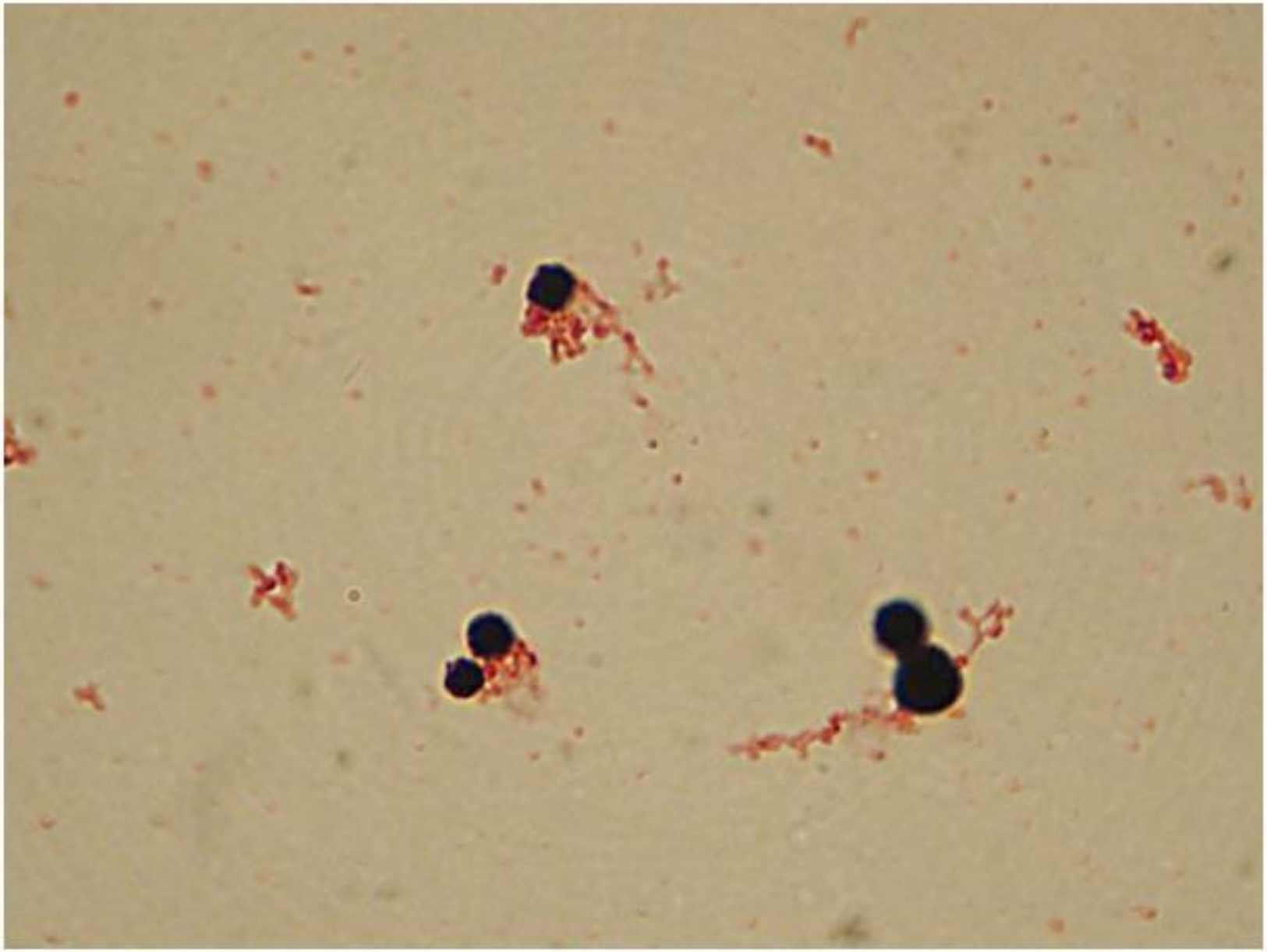
Median rhomboid glossitis

Cryptococcus spp. Morphology

- Do not form pseudo- or true hyphae, except for *Cryptococcus neoformans*, which rarely forms them
- Urease positive
- *Cryptococcus neoformans* is the only common, medically important yeast to produce phenol oxidase, colonies grown on **birdseed agar** (which contains niger or thistle seeds) turn dark brown in 2-5 day of incubation



Cryptococcus neoformans 1000x stained with Calcofluor.



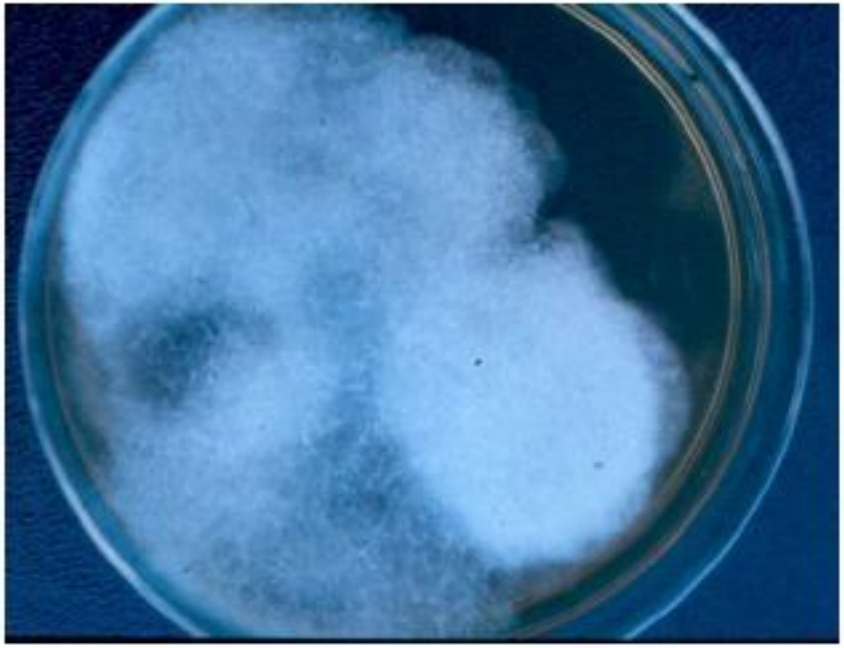
***Cryptococcus neoformans* in blood culture; gram stain.**

Diagnosis of Fungal Infection

- **Microscopy** – direct staining of fungi in sections can distinguish between yeasts and molds
- **Culture** – can lead to diagnosis of the exact species. *Candida* can be grown in blood cultures but *Aspergillus* cannot
- **Serology** – direct detection of fungal antigens in serum samples. ELISA to detect galactomannan (Platelia – BioRad) or detection of ß-d-glucan, does not detect *Cryptococcus* spp or zygomycetes
- **Radiography** – direct observation of patients to spot characteristic signs of infection, e.g. halo signs, cavities
- **PCR** – assays target fungal ribosomal operon, nucleic acid extraction from blood or BAL. Potentially very sensitive but still no standardised tests

(Hope *et al.* 2005)

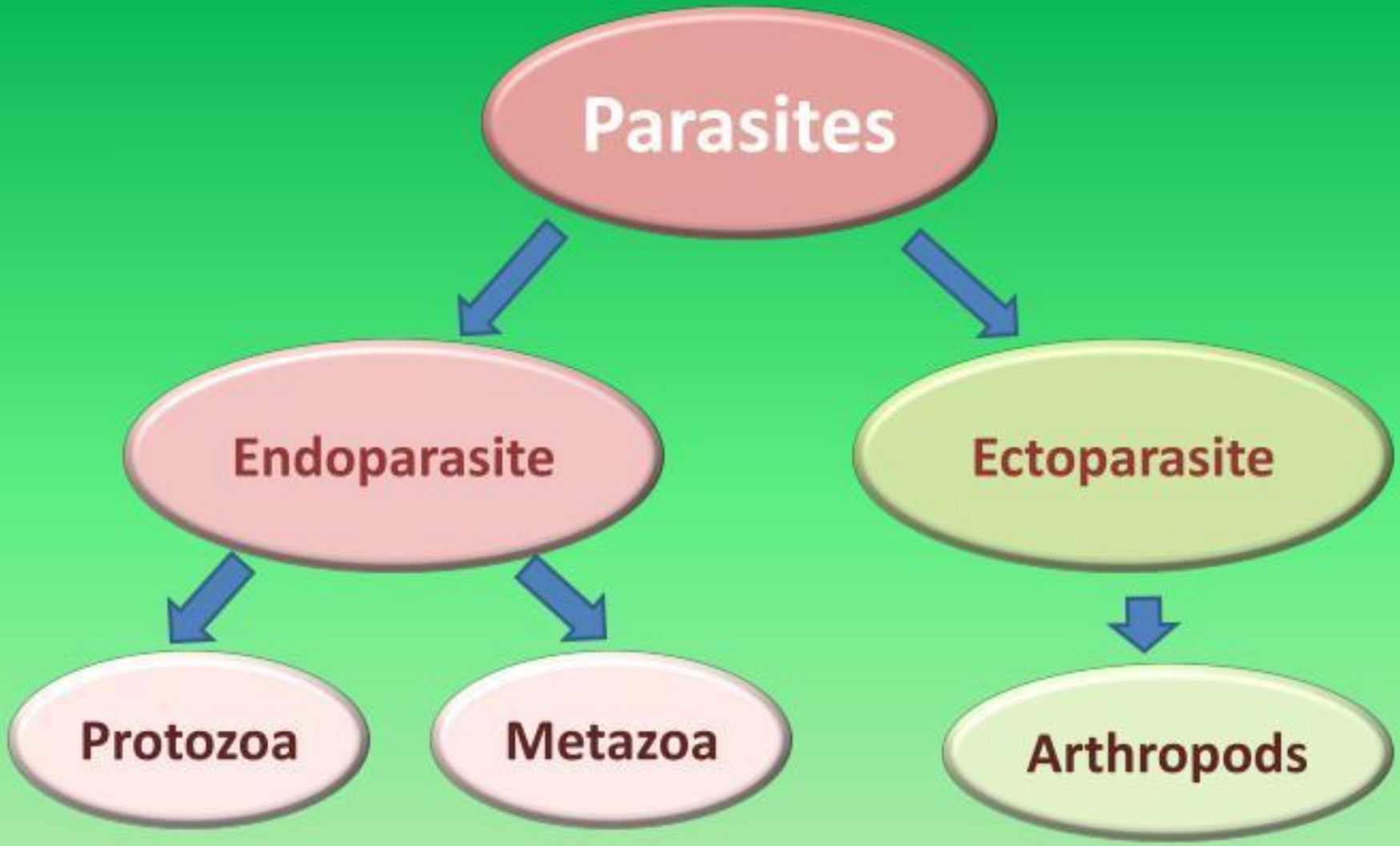
Mucor



Antifungal drugs

- Selective toxicity is very limited in antifungal drugs because fungi like human cells are eukaryotic.
- They are:

1) **Amphotericin B:**
   - Binds to the ergosterol in the fungal cell membrane.
   - Used in severe fungal infections.
   - Nephrotoxic.
2) **Flucytosine:**
   - Inhibit fungal DNA synthesis.


Parasites
Endoparasite
Ectoparasite
Protozoa
Metazoa
Arthropods

Protozoa Characteristics

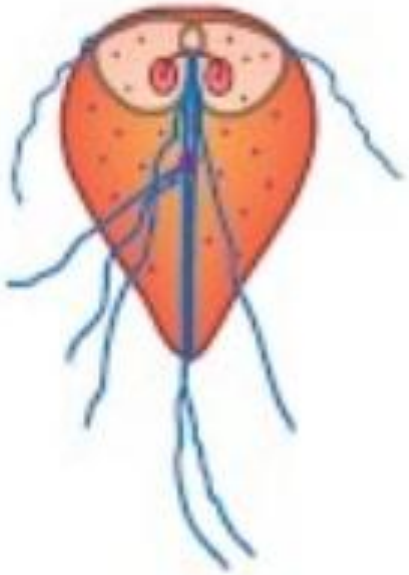
Cyst

Trophozoite

- Protozoa are, eucaryotic organisms.
- Most protozoa are unicellular and free-living; found in soil and water.
  - Most protozoa are more animal-like than plant-like.
  - All protozoal cells possess a variety of eucaryotic structures/organelles.
  - Protozoa cannot make their own food; they ingest whole algae, yeasts, bacteria, and smaller protozoa for nutrients.

Giardia

Cyst

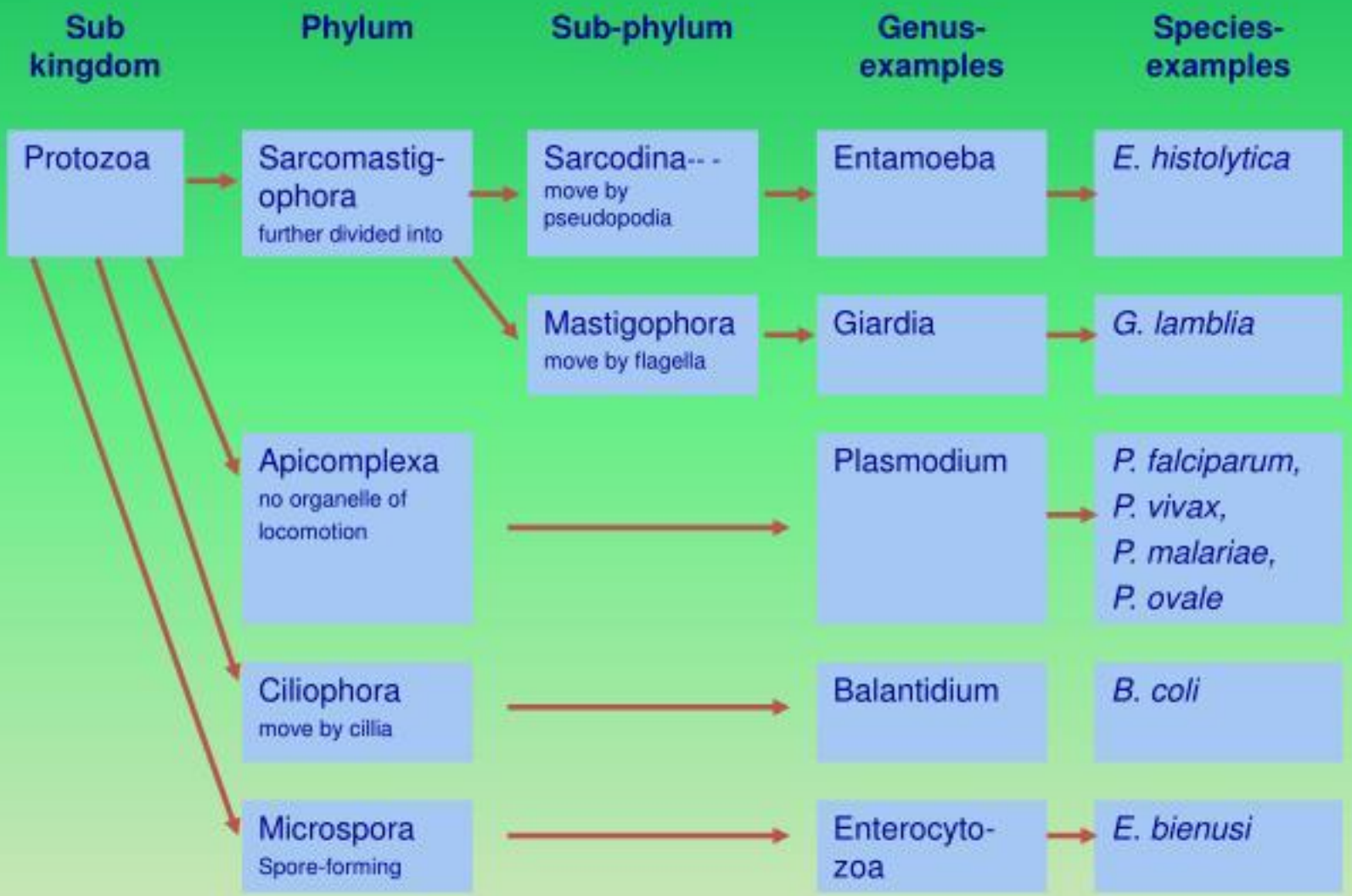


Trophozoite

Protozoa

- Some protozoa are parasites.
- Parasitic protozoa break down and absorb nutrients from the body of the host in which they live.
- Many parasitic protozoa are pathogens, such as those that cause:
  - Malaria,
  - Giardiasis,
  - African sleeping sickness,
  - Amebic dysentery

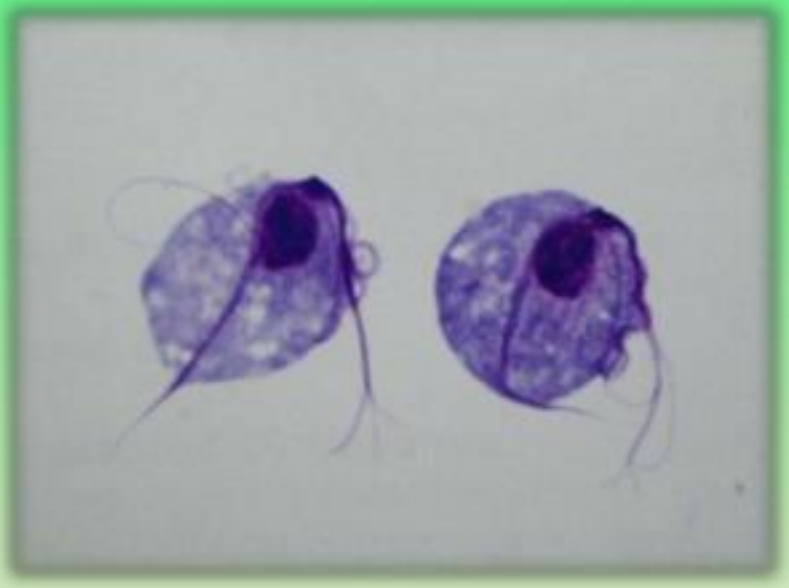
Classification of Protozoa



- **Intestinal**
  - Amebiasis ..... *Entamoeba histolytica*
  - Giardiasis .....*Giardia lamblia*
  - Balantidiasis ..... *Balantidium coli*
  - Crytosporidosis ..... *Cryptosporidium parvum*
  - Cyclosporiasis ..... *Cyclospora cayetanensis*
- **Genitourinary tract**
  - Trichomoniasis ..... *Trichomonas vaginalis*
- **Blood and Tissue**
  - Malaria ..... Plasmodium spp
  - Meningoencephalitis ..... *Naegleria fowleri*
  - Toxoplasmosis ..... *Toxoplasma gondii*..... **(Eye)**
- **Cardiovascular system**
  - African Sleeping Sickness ..... *Trypanosoma brucei*..... **(CNS)**
  - Chagas Disease ..... *Trypanosoma cruzi*
- **Skin and mucous membrane**
  - Visceral leishmaniasis(Kala-azar) ... *Leishmania donovani*
  - Cutaneous leishmaniasis ..... *Leishmania topica/braziliensis*

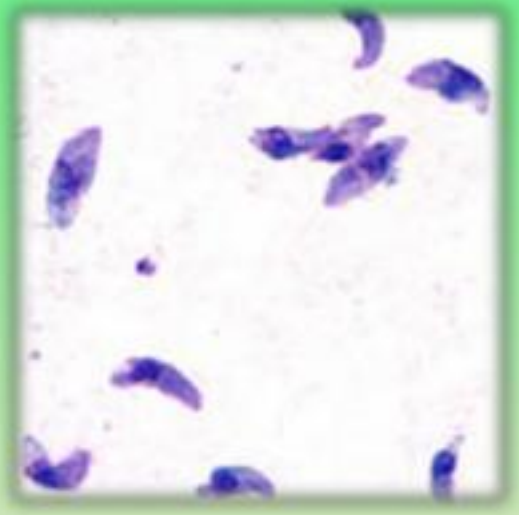
Medically important Protozoan Diseases

- **Genitourinary tract infections**
  - Trichomoniasis
    - ✓ By *Trichomonas vaginalis*
    - ✓ A flagellate that has no cyst form
    - ✓ Sexually transmitted disease, STD
    - ✓ Symptomatic in females and Asymptomatic in males
    - ✓ Saline wet mount examination of vaginal or urethral discharge – motile trophozites



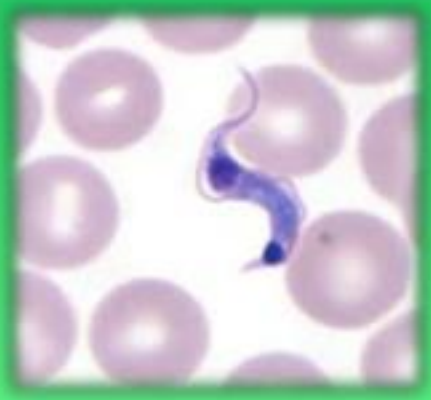
Medically important Protozoan Diseases

- **Protozoal infections of the eyes**
  - Amebic Eye infection
  - Toxoplasmosis
    - ✓ By *Toxoplasma gondii* , intracellular sporozoan
    - ✓ Ingestion of raw or undercooked meat containing the cyst
    - ✓ Typically diagnosed by Immuno-Diagnostic Procedures, IDPs
    - ✓ Stained biopsy

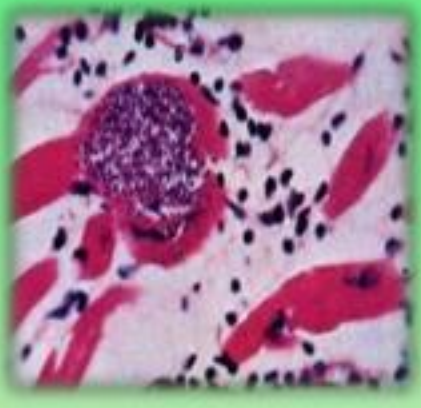


Medically important Protozoan Diseases

- **Cardiovascular system infections**
  - **African Sleeping Sickness (African trypanosomiasis)**
    - ✓ By *Trypanosoma brucei,* Hemoflagellates
    - ✓ Transmitted by Tsetse flies
    - ✓ Diagnosed by observing trypomastigotes in blood, lymph node aspirates or CSF

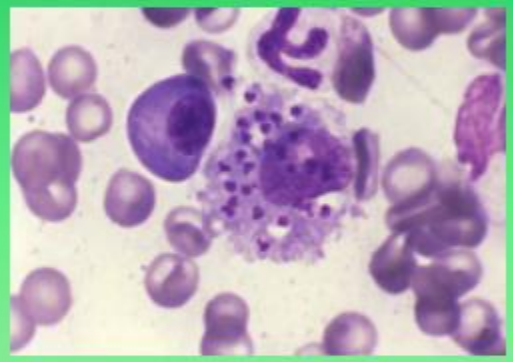


  - **Chagas Disease (American trypanosomiasis)**
    - ✓ By *Trypanosoma cruzi*, Hemoflagellates
    - ✓ Transmitted by infected reduviid bugs, or blood transfusion
    - ✓ Diagnosed by observing trypomastigotes in blood, lymph node biopsies or tissue



Medically important Protozoan Diseases

- **Skin and mucous membrane**
  - Visceral leishmaniasis(Kala-azar)
    - ✓ *Leishmania donovani*
  - Cutaneous leishmaniasis
    - ✓ *Leishmania topica/braziliensis*



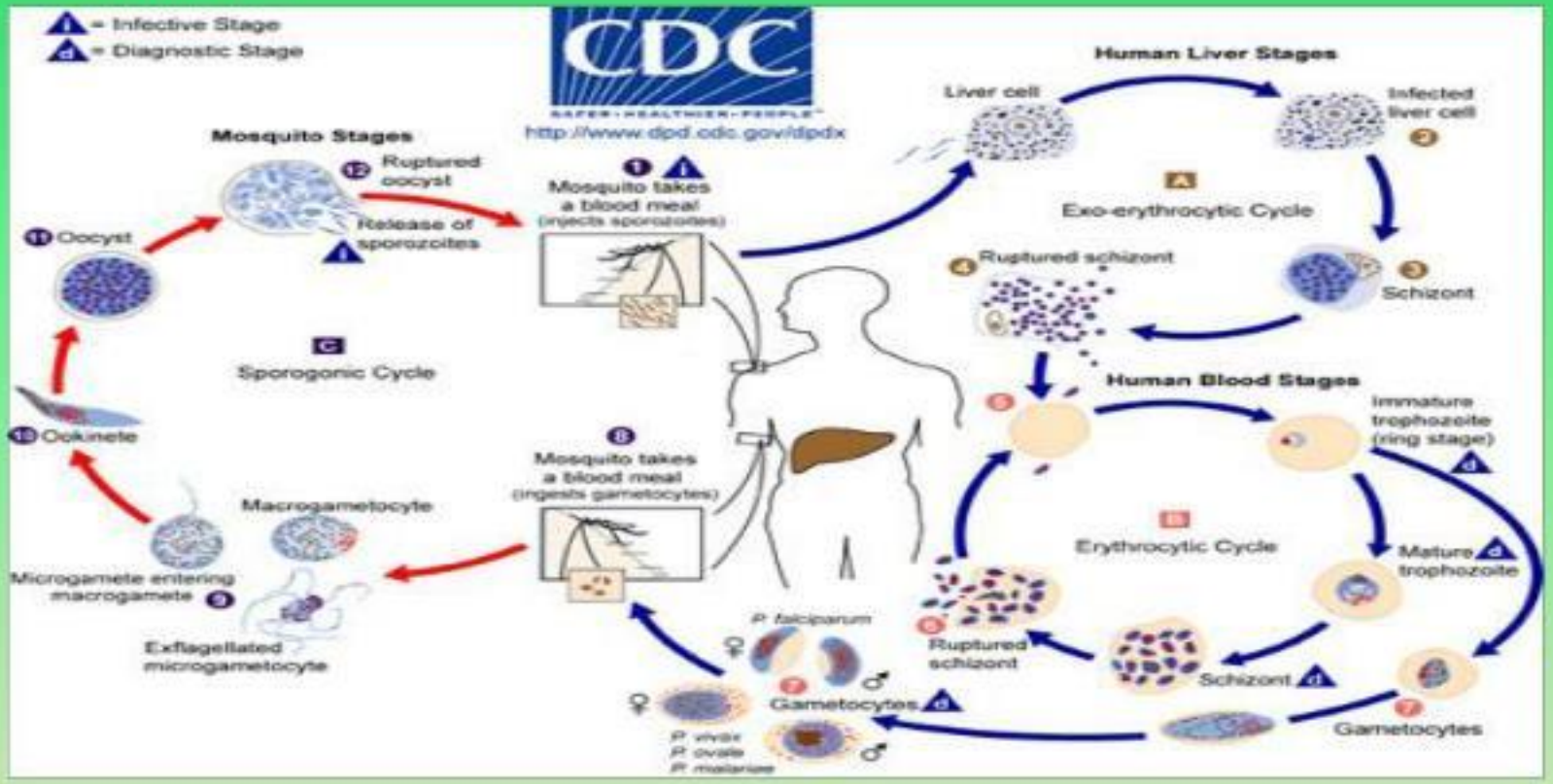
- *Leishmania* spp.
  - ✓ Tissue flagellates
  - ✓ Usually transmitted by sandfly
  - ✓ Many animal reservoirs
  - ✓ Diagnosis usually by microscopic identification of non-motile amastigote form inside macrophages
  - ✓ Some diagnosis by IPDs

Examples of protozoal infections diagnosed by examining stained blood smears

Infection	Observation required for Diagnosis
• African trypanosomiasis	• Extracellular trypomastigotes
• American trypanosomiasis	• Extracellular trypomastigotes
• Babesiosis	• Intraerythrocytic protozoa
• Malaria	• Intraerythrocytic protozoa

Medically important Protozoan Diseases

- **Blood and Tissue**
  - Malaria



Giardia lamblia

- Also known as – G. intestinalis / Lamblia intestinalis.
- **HISTORY** :
  - First seen by Antonie Von Leeuwenhoek by examining his own stool.

MORPHOLOGY

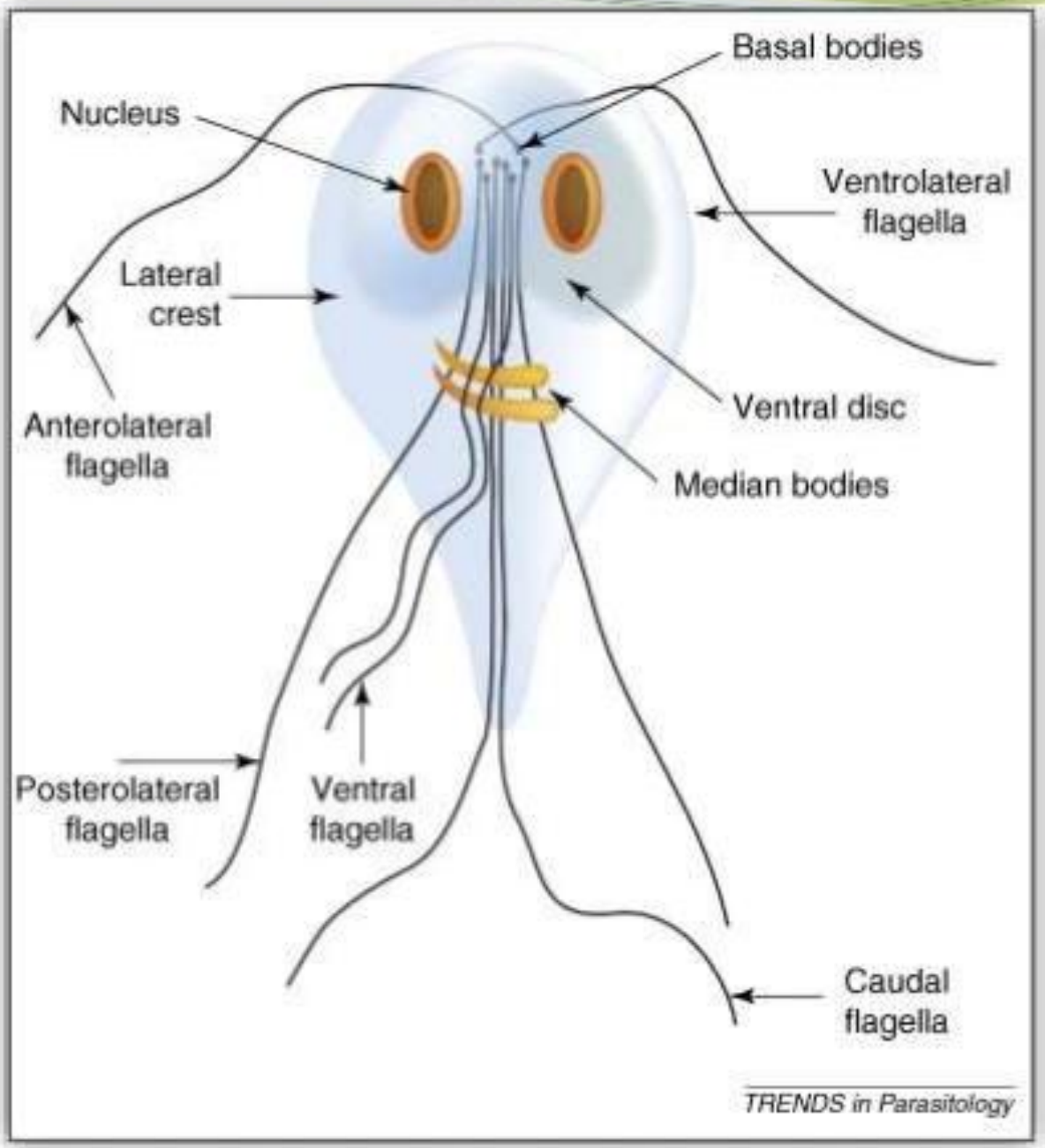
- It exists in two forms –

1) Trophozoit (Vegetative form)

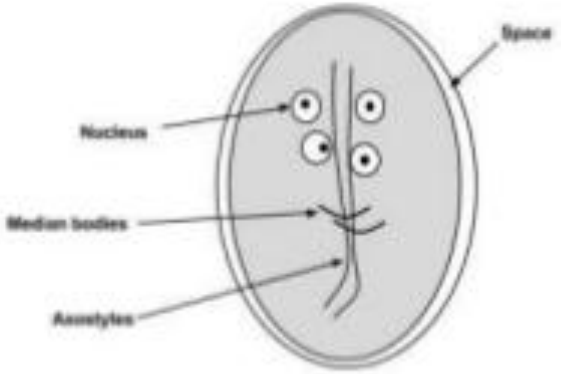
2) Cyst (Infective form)

TROPHOZOITE

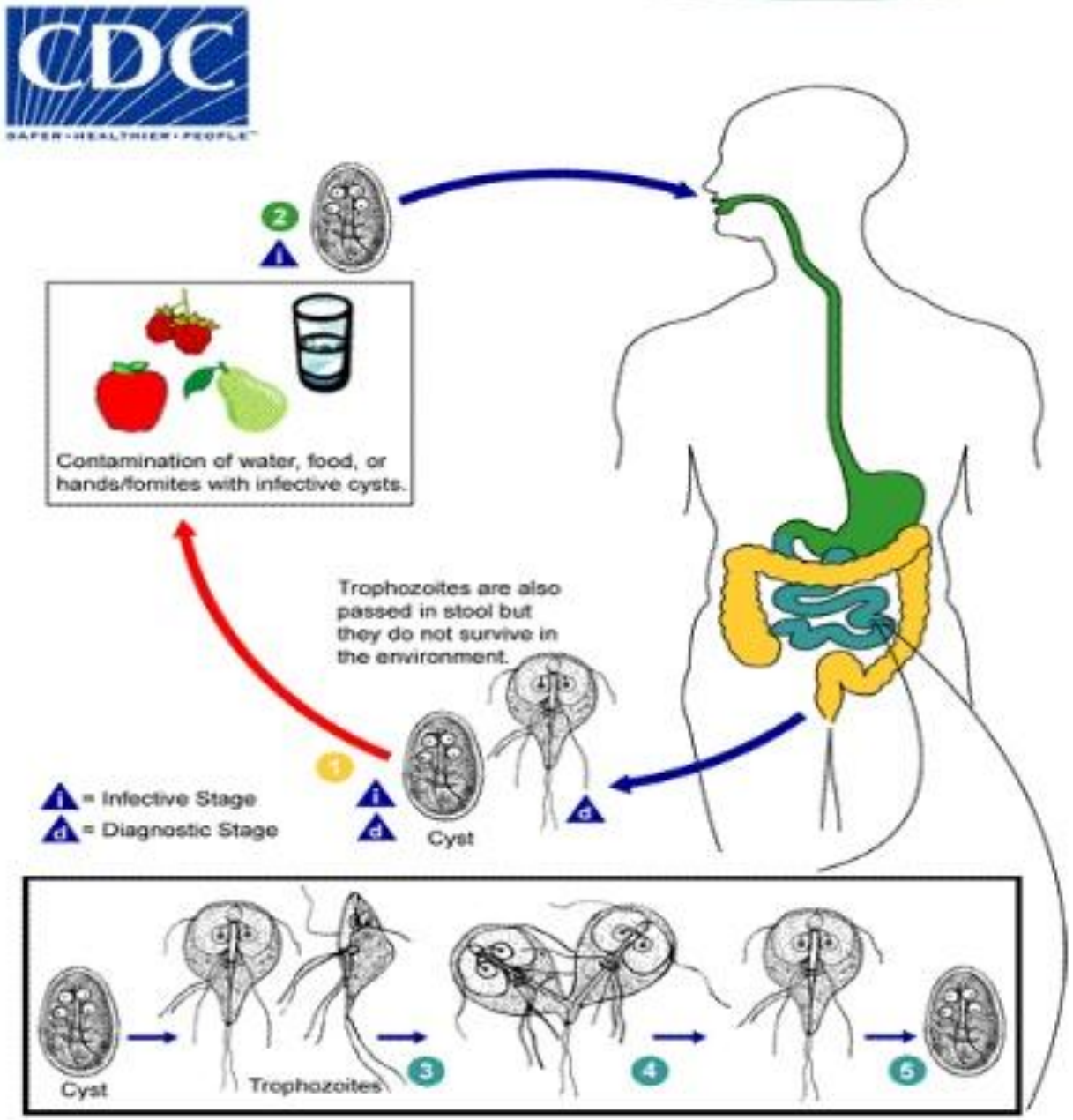
- Tennis racket or heart shaped or pyriform shaped.
- Dorsal surface – convex
- Ventral surface – concave & having sucking disk (for attachment)
- 14 μm x 7μm x 4μm
- Anterior end – broad & rounded
- Posterior end – tappers to a sharp point
- Bilaterally symmetrical :
  - Nuclei – 1 pair
  - Flagella 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

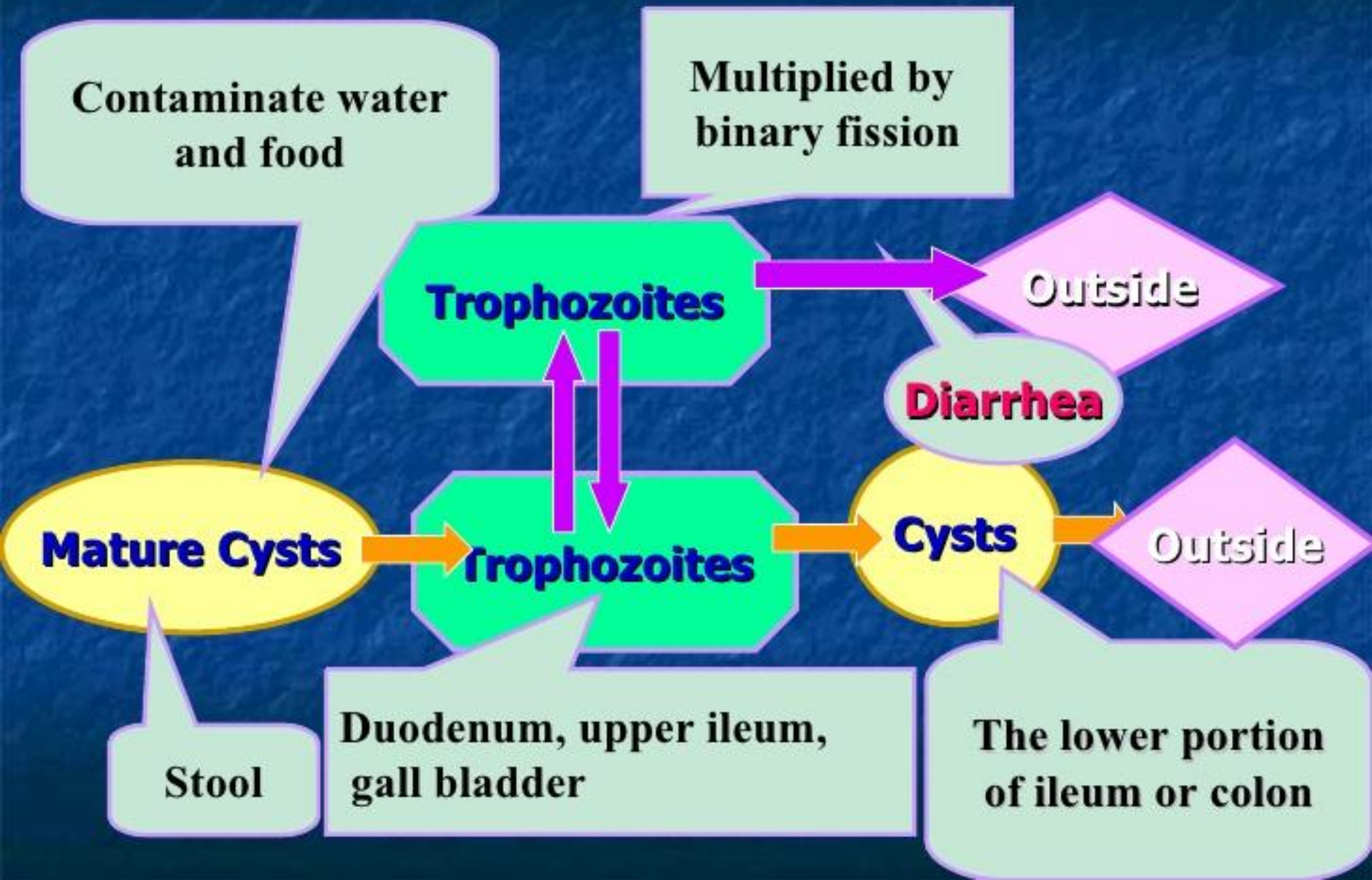
CYST



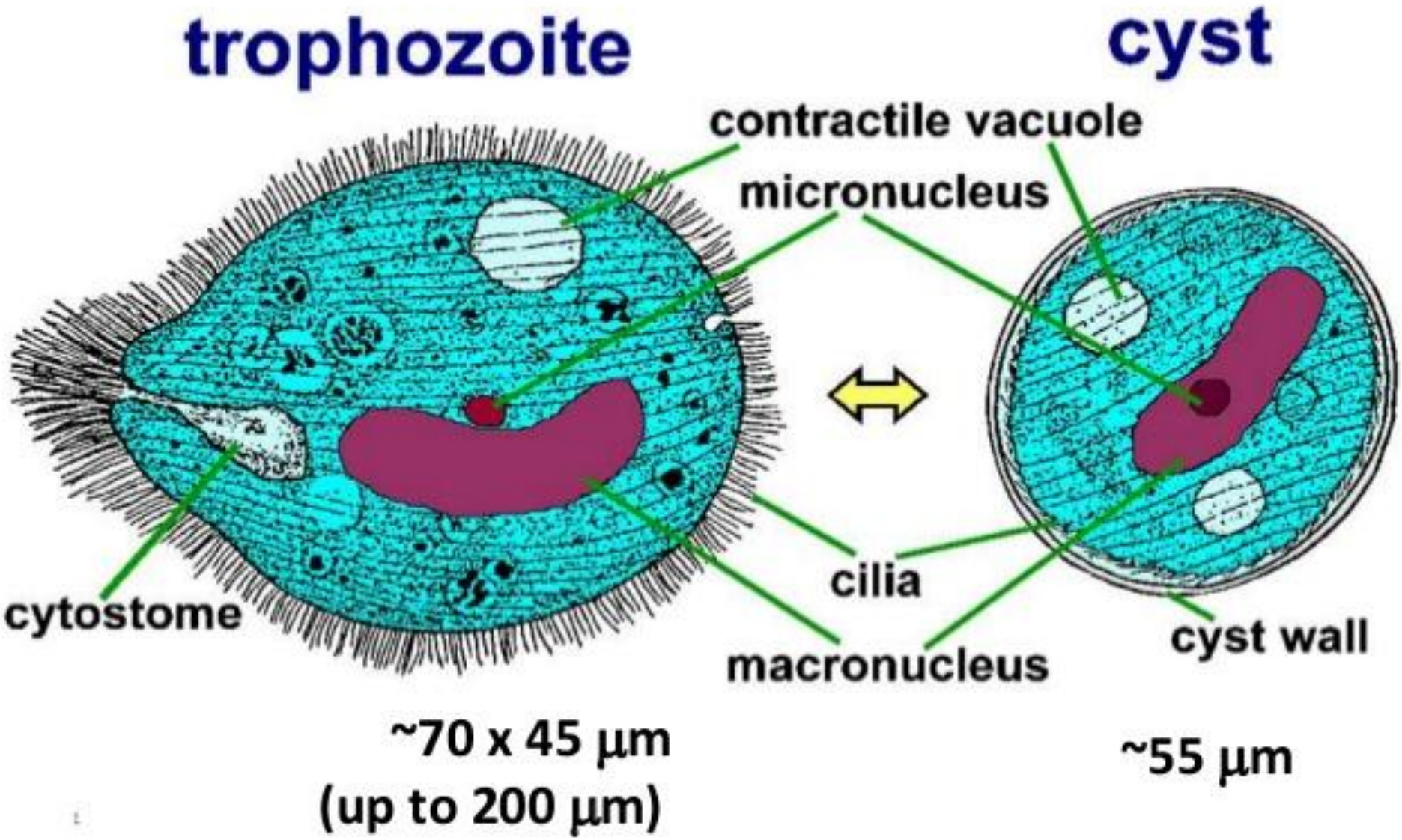
- Round or oval in shape.
- Surrounded by hyaline cyst wall.
- 12μm x 7μm.
- Axostyle – diagonally placed, form a deviding line within cyst.
- 4 nuclei – clustered at one end or at opposite poles (each pairs).
- Remnants of flagella and margins of the sucking disc may be seen inside the cytoplasm of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

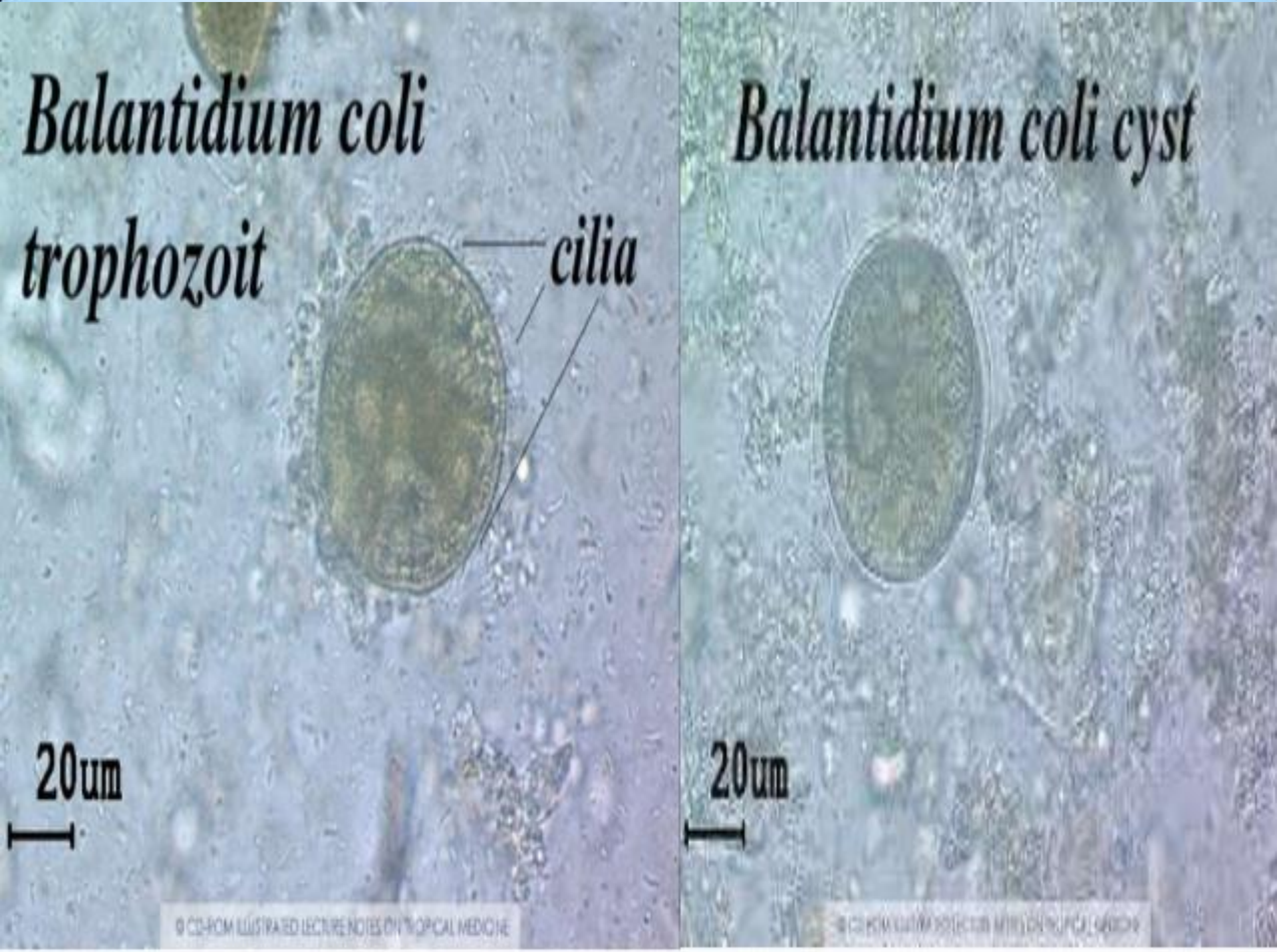

CDC
SAFER·HEALTHIER·PEOPLE
2
1
Contamination of water, food, or hands/fomites with infective cysts.
Trophozoites are also passed in stool but they do not survive in the environment.
1
i
d
Cyst
i = Infective Stage
d = Diagnostic Stage
Cyst
Trophozoites
3
4
5

Life cycle of *Giardia lamblia*



Balantidium coli




Balantidium coli
trophozoit
cilia
20um
Balantidium coli cyst
20um

Balantidium coli

- Swine (pigs) are reservoir host.

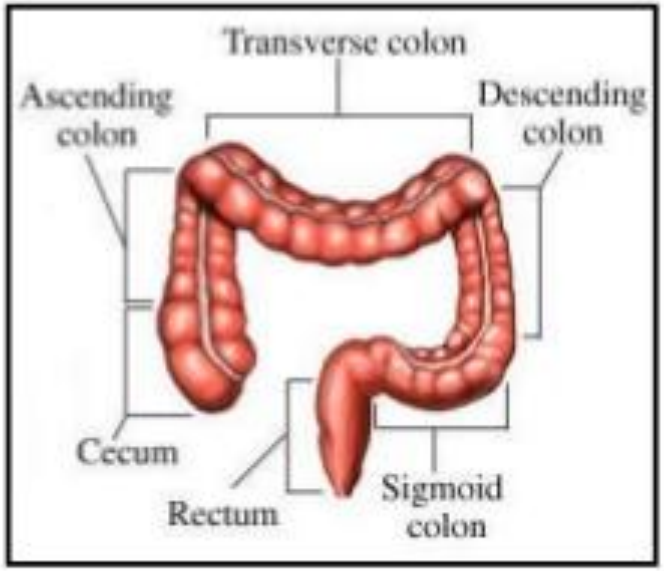
- Causes disease called Balantidiasis (Ciliary dysentery).

- **Mode of transmission:**
  - By feco-oral route.
  - Contaminated water is the most common mechanism of transmission.

Balantidium coli

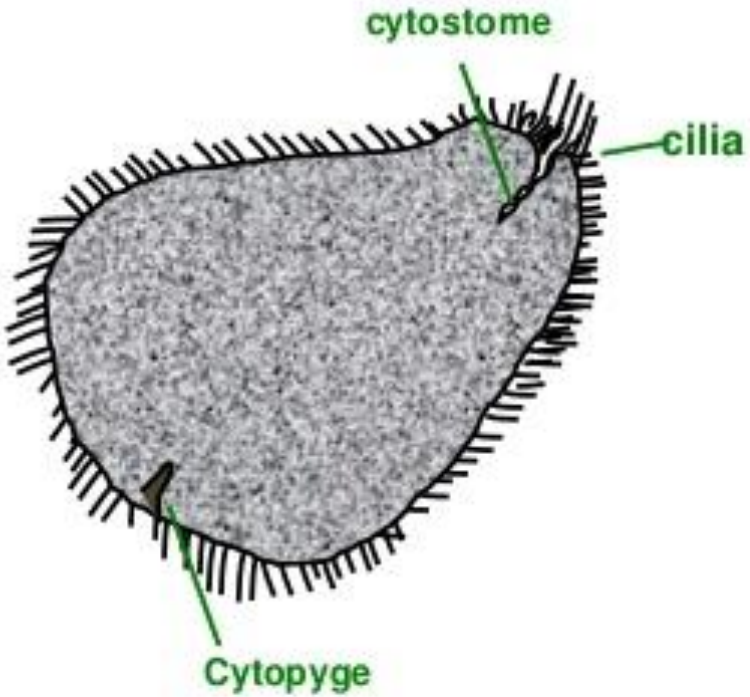
➢ **Habitat and location:**

Balantidium coli lives in the cecum and colon (large intestine) of humans, pigs, rats and other mammals.

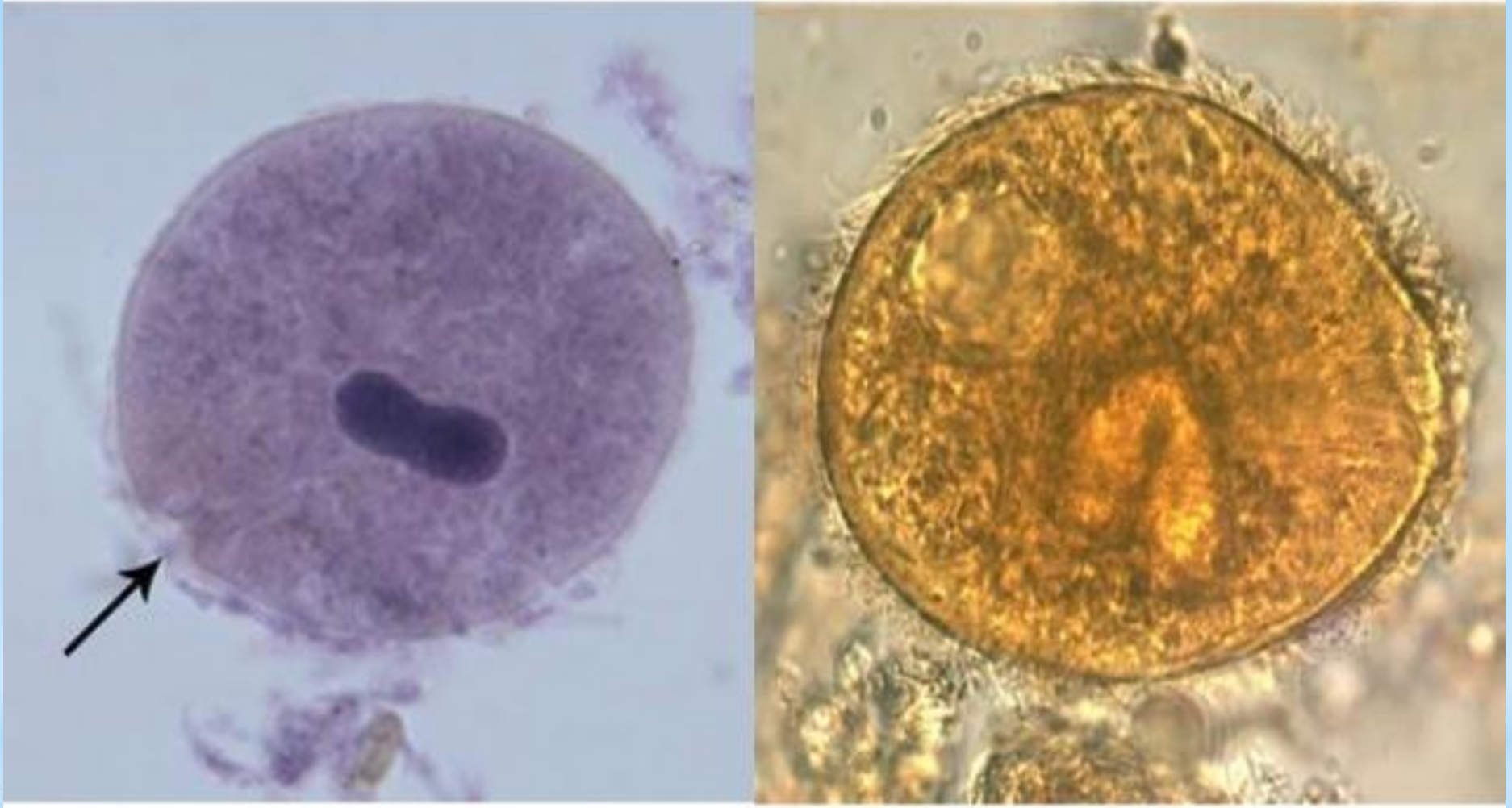


Balantidium coli

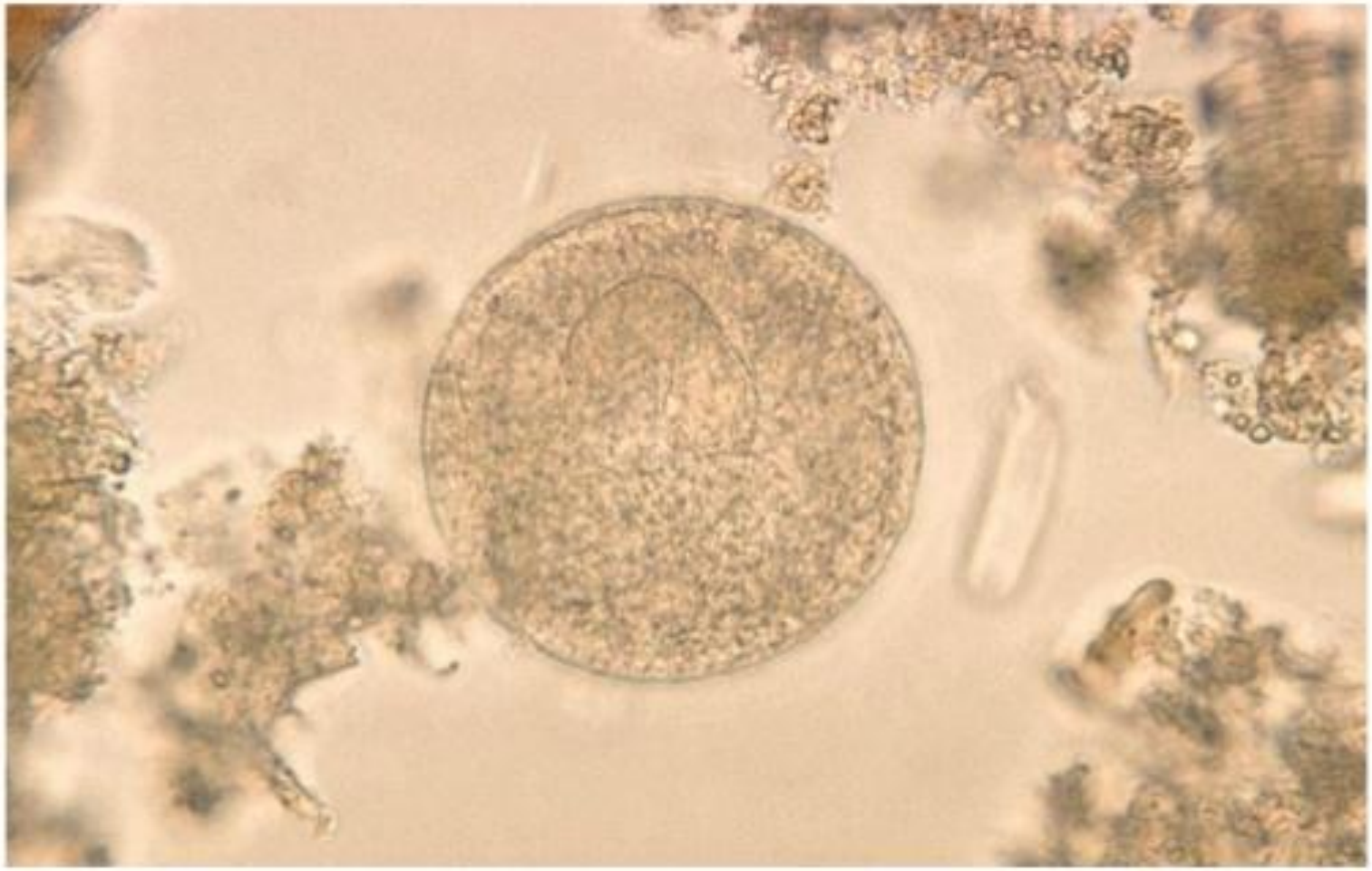
- This organism has two stages, trophozoite and cyst.
- Trophozoite, is the largest of the protozoa parasitizing human (300 um), it is shape like a sac, balantidium means little bag.
- It is ovoidal, greenish-gray in color.
- It is covered with short cilia which are constantly in motion during life.



- The anterior end is conical and the posterior end broadly rounded.



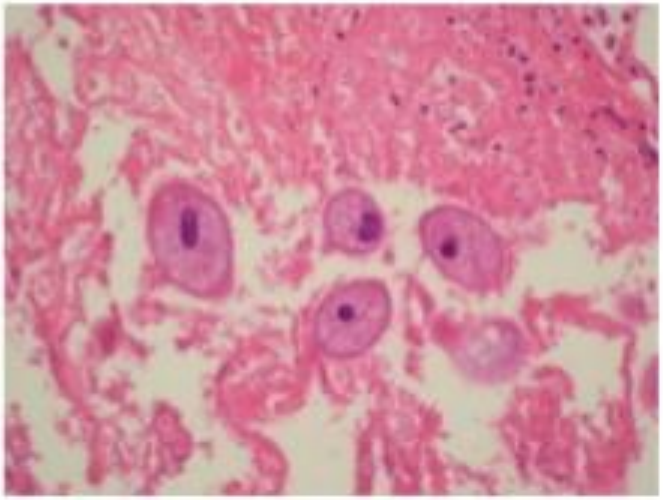
Trophozoite

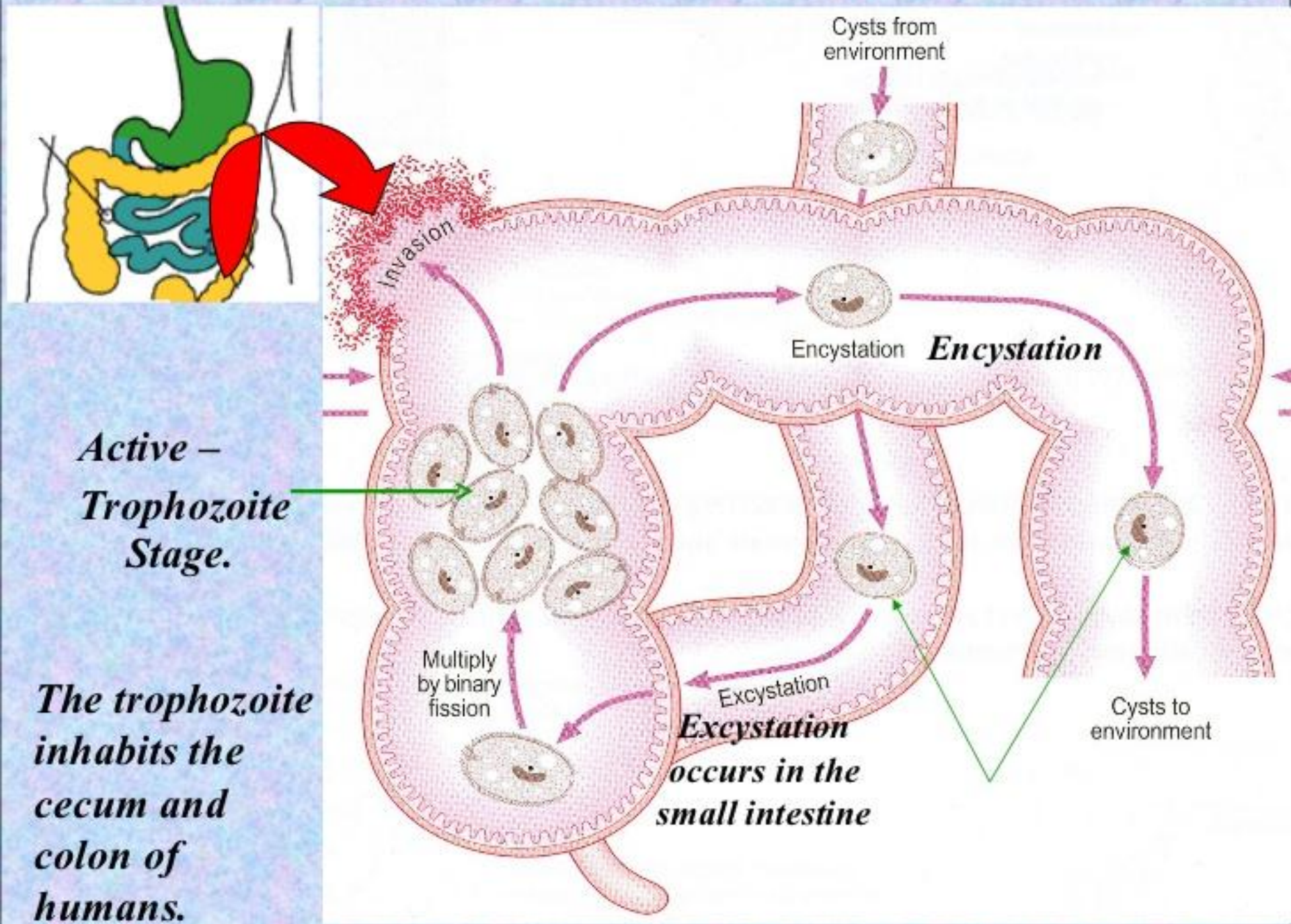


Cyst

Clinical Signs

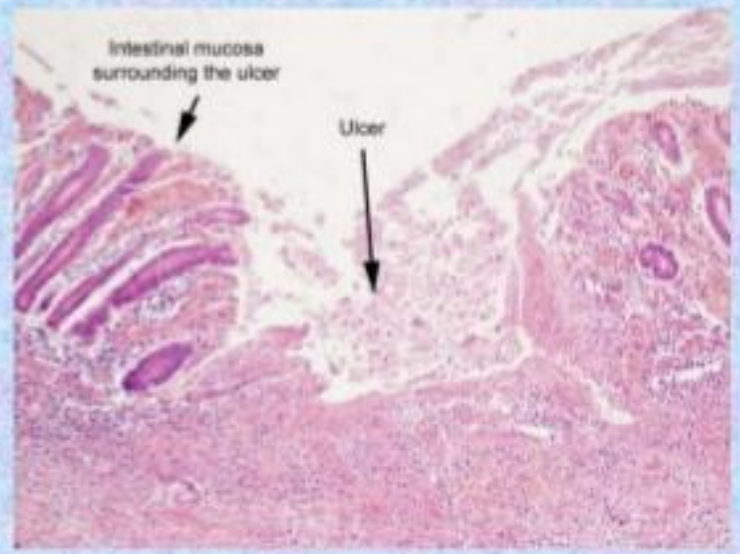
- Symptoms of balantidiasis are similar to those seen in entamebiasis. liver, lung and brain abscesses are not found.
- Ulceration of the gut wall.
- Dysentery or profuse diarrhea.
- The resultant erosion of the intestinal mucosa produces varying degrees of irritation and injury, leading to nausea, vomiting, diarrhea, dysentery, and abdominal colic.




Cysts from environment
Invasion
Encystation
Encystation
Active – Trophozoite Stage.
Multiply by binary fission
Excystation
Excystation occurs in the small intestine
Cysts to environment
The trophozoite inhabits the cecum and colon of humans.

Balantidium coli: Pathology

Trophozoites are tissue invaders. They secrete proteolytic enzymes (Hyaluronidase) which digest the epithelium of the large intestine.



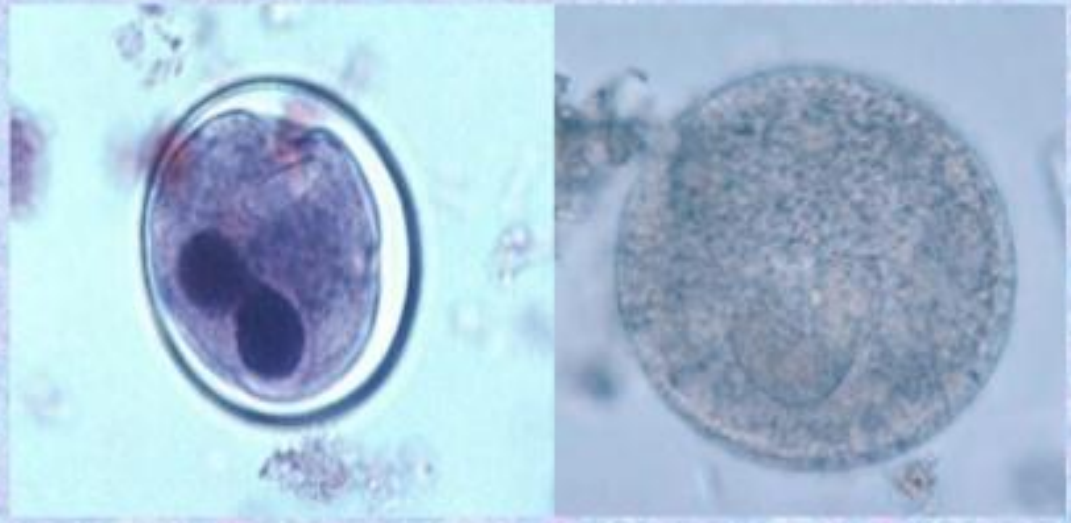
Ulceration results in bleeding and secondary bacterial infection.

Perforation of the large intestine has occurred in some fatal cases.

Laboratory diagnosis

1. Examination of stool samples, looking for trophozoites and cysts, which are readily identified because of their large size.

Trophozoite



Cyst

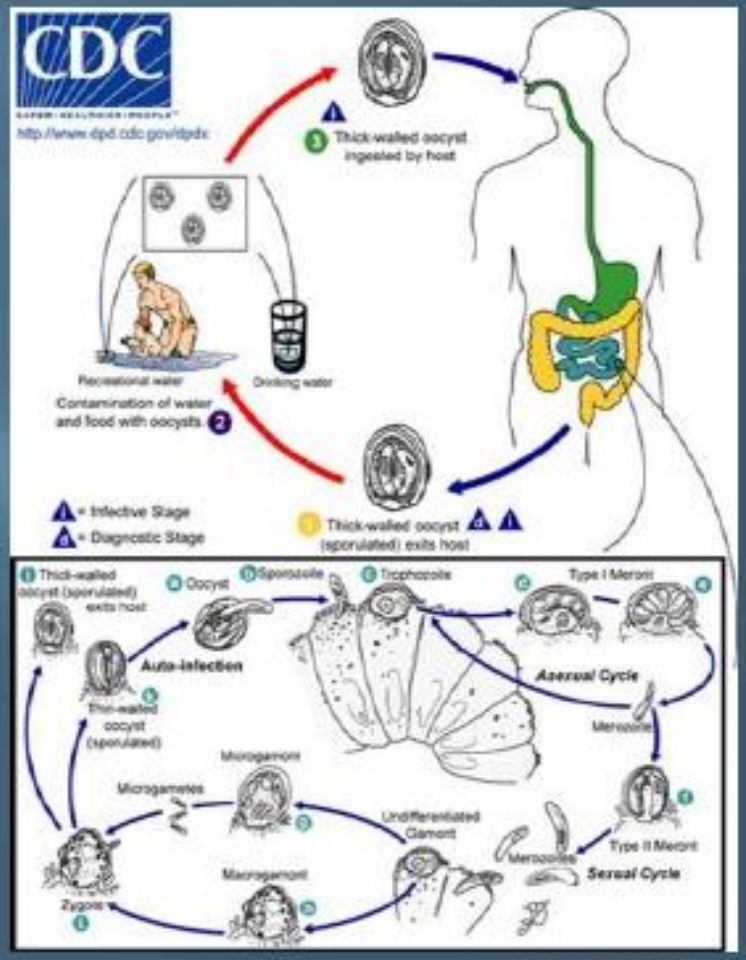
Cryptosporidium

- Protozoan
- Phylum: Apicomplexa
- Class: Sporozoasida
- Order: Eucoccidiida
- Family Cryptosporiidae
- Genus: *Criptosporidium*
- Species: ***parvum***, *muris, meleagridis, felis, etc.*

waterfilterreview.com

Etiology

- Buries into intestinal lining of the gut
  - Goes through Life Cycle
- Alters osmotic pressure
  - Diarrhea
- Atrophy of intestinal villi
  - Alters uptake of fluids, electrolytes, and nutrients
  - Malabsorption syndrome



CDC DPDx Library

Symptoms

- Symptoms (2 to 10 days after infection and can last up to 30 days)
  - Diarrhea
  - Stomach cramps
  - Dehydration
  - Nausea
  - Vomiting
  - Fever
  - Weight loss
  - Sometimes no symptoms are seen

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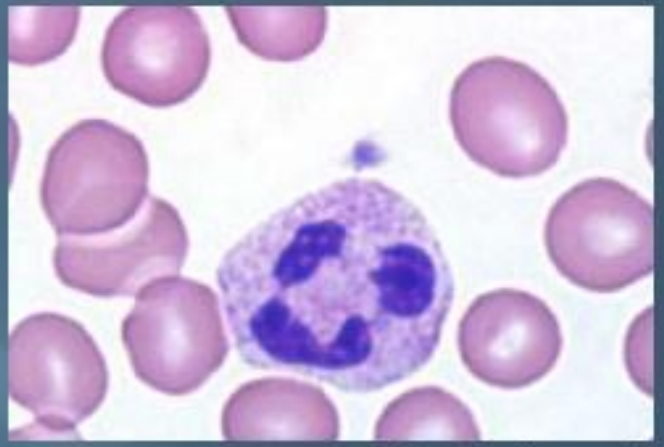
Innate Immune Response

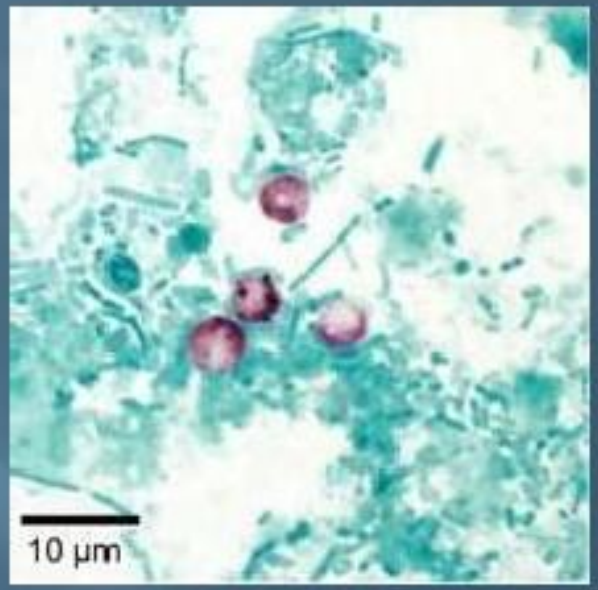
- White Blood Cells Phagocytize Parasites
  - Segmented neutrophils
  - Macrophages
  - Lymphocytes
  - Eosinophils



labmed.hallym.ac.kr

Modified Acid-Fast Stain

- Visualization of Oocytes
  - Light pink to dark red
  - Can also visualize sporozoites
- Relatively High Sensitivity and Specificity
- Irregular Staining
  - cause "ghost" oocysts



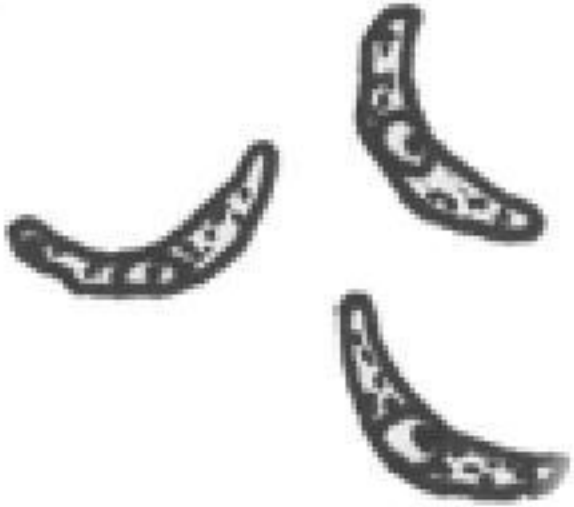
CDC DPDx Library

Modified Acid Fast Stain

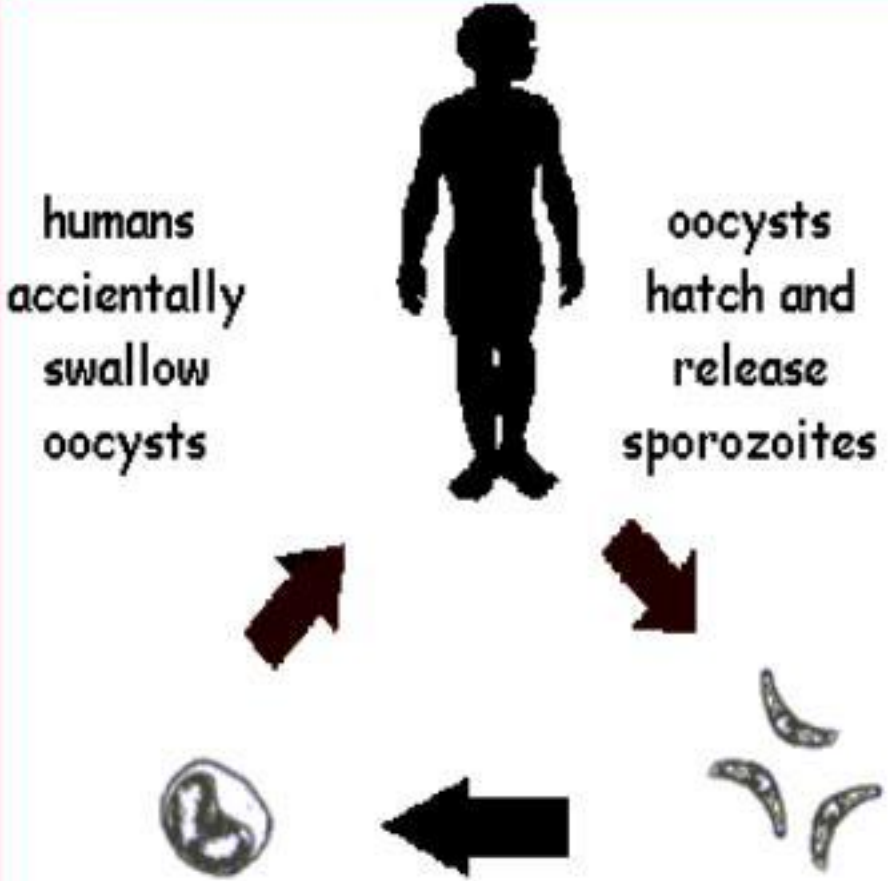
Cryptosporidiosis is spread by faecal-oral route.

A. Oocyst

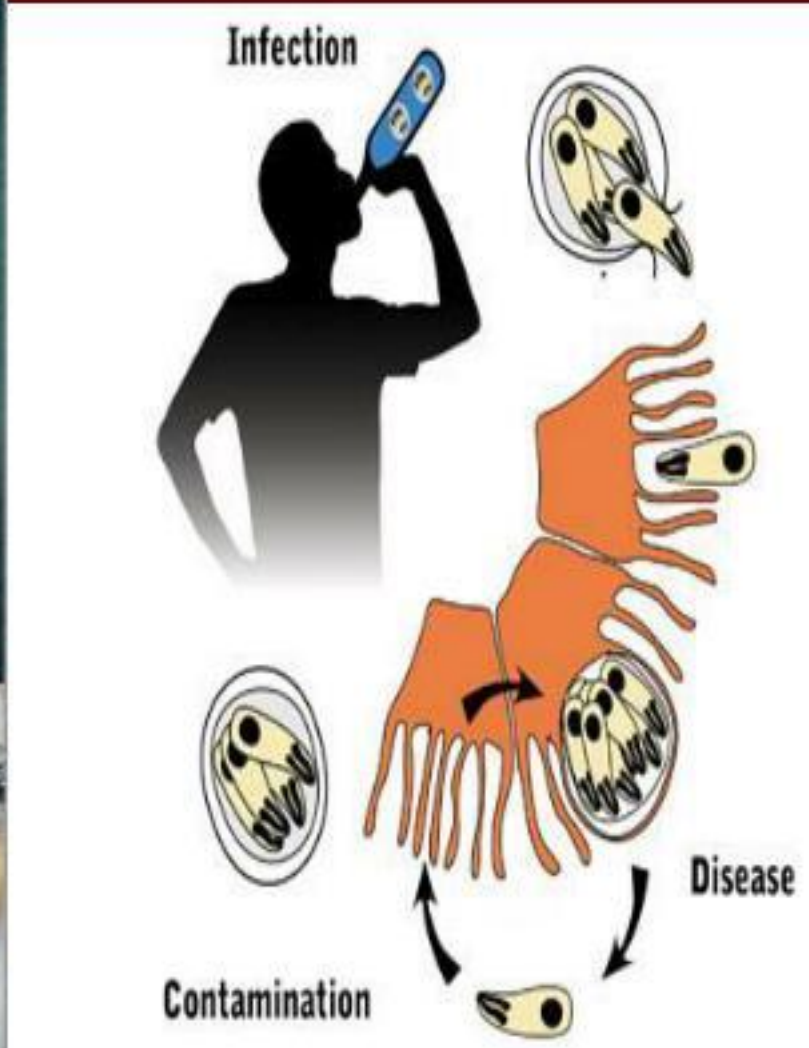
B. Sporozoites



A. & B. over 4000 times bigger than normal



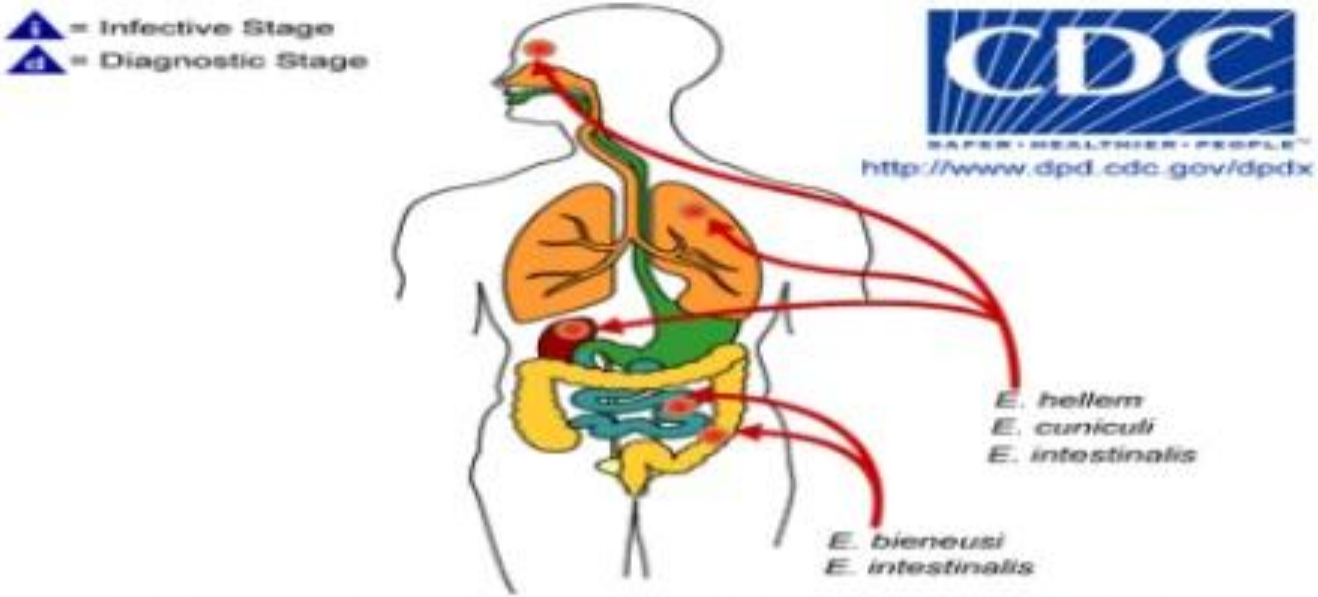
Cryptosporidium can hitch a ride on food-or in water-causing diarrhea and even death in vulnerable individuals.



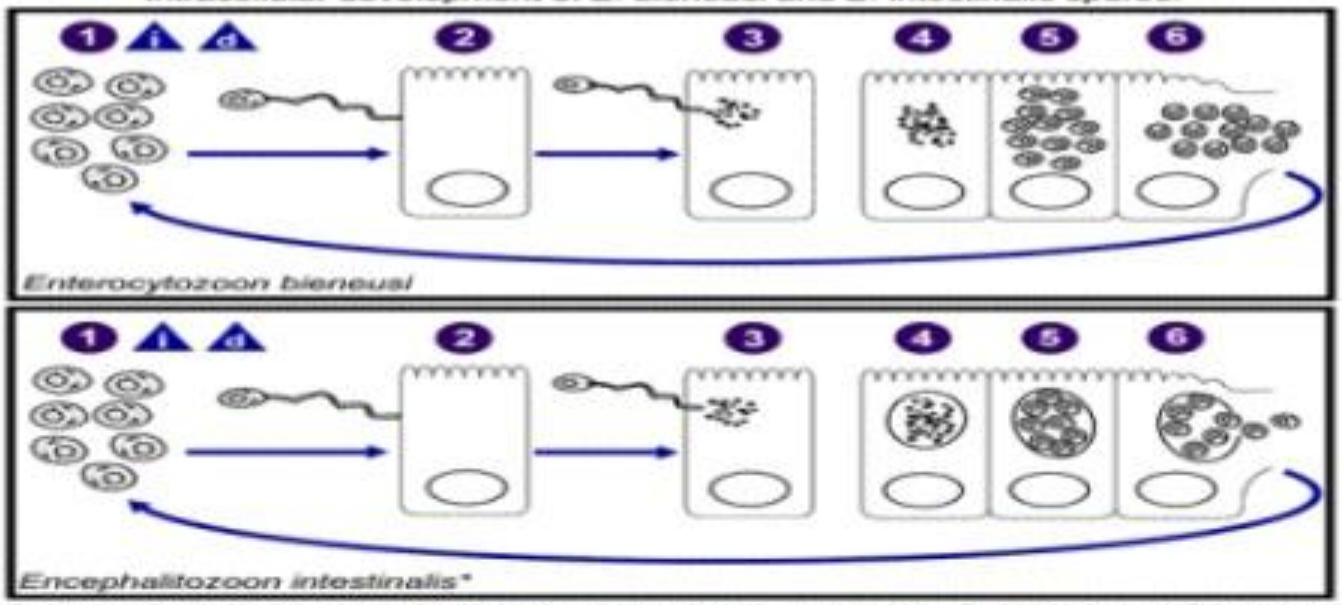
MICROSPORA

- Microsporidia
- 7 genera
- Enterocytozoon (*E.bieneusi, E.intestinalis*), Encephalitozoon, Nosema, Pleistophora, Thelohanea, Trachipleistophora & Vittaforma
- Causes microsporidiosis especially in AIDS cases causing persistent diarrhoea, abdominal cramps, nausea, malabsorption

MICROSPORA

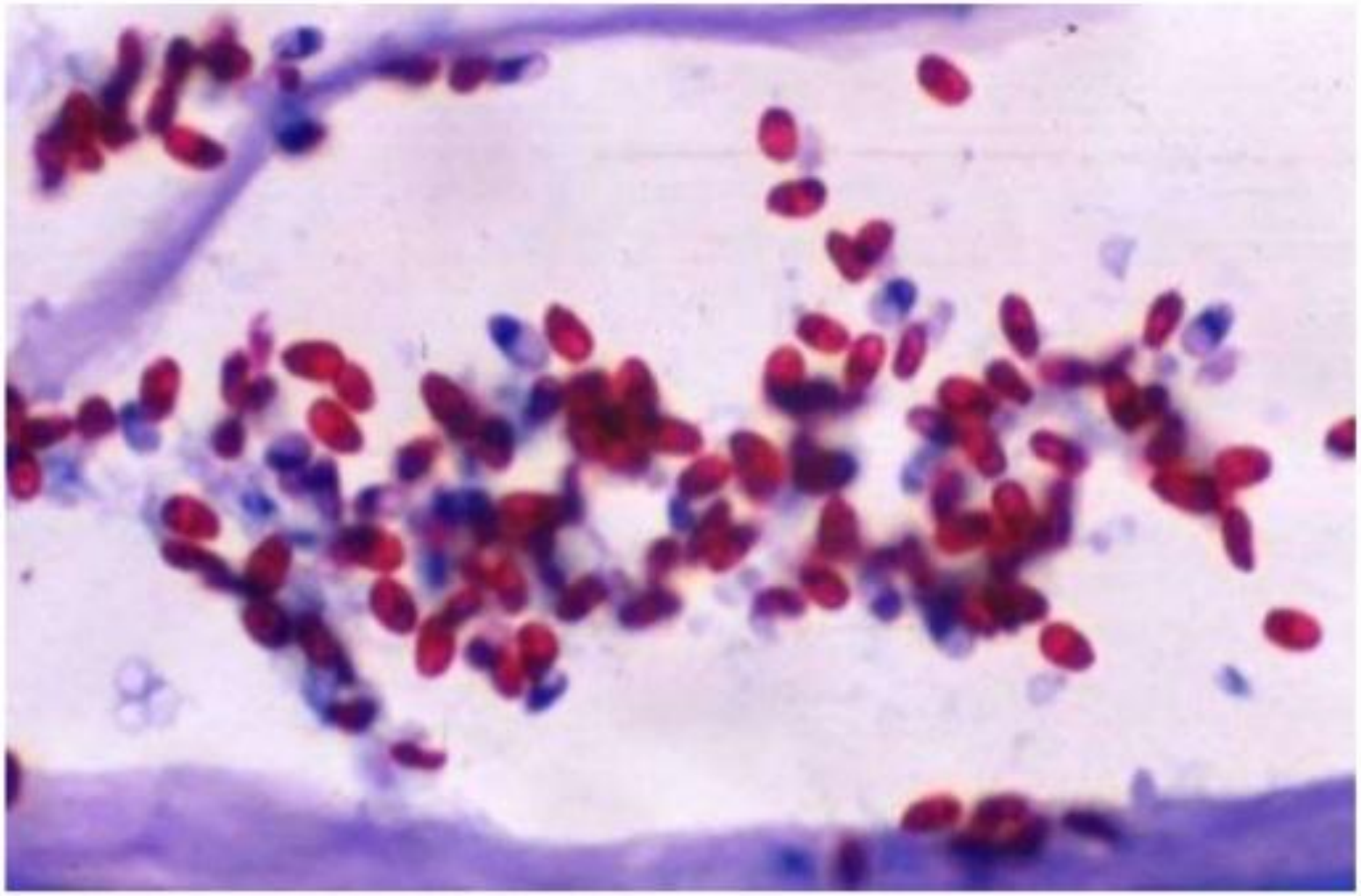


Intracellular development of *E. bieneusi* and *E. intestinalis* spores.

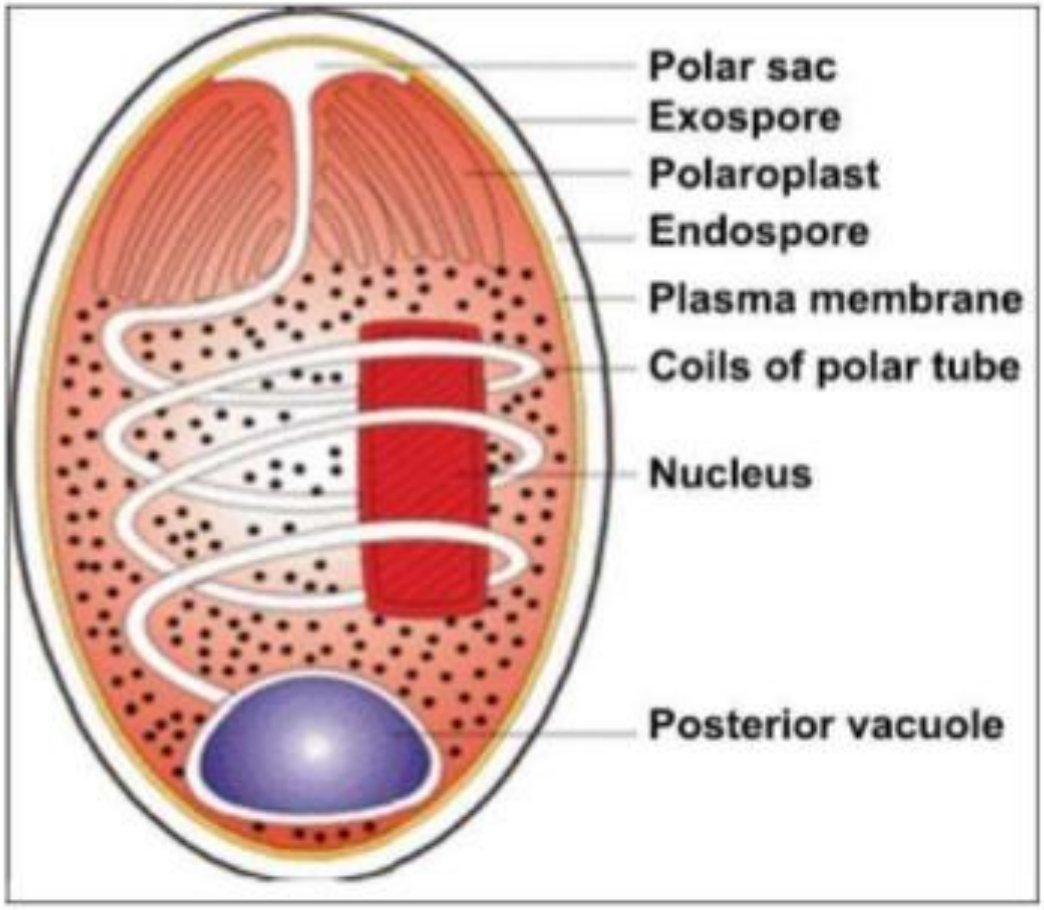


*Development inside parasitophorous vacuole also occurs in *E. hellem* and *E. cuniculi*.

MICROSPORIDIA



MICROSPORIDIA



Entamoeba histolytica (Amebae).

AMEBIASIS (**amebic dysentery**, **amebic hepatitis**)

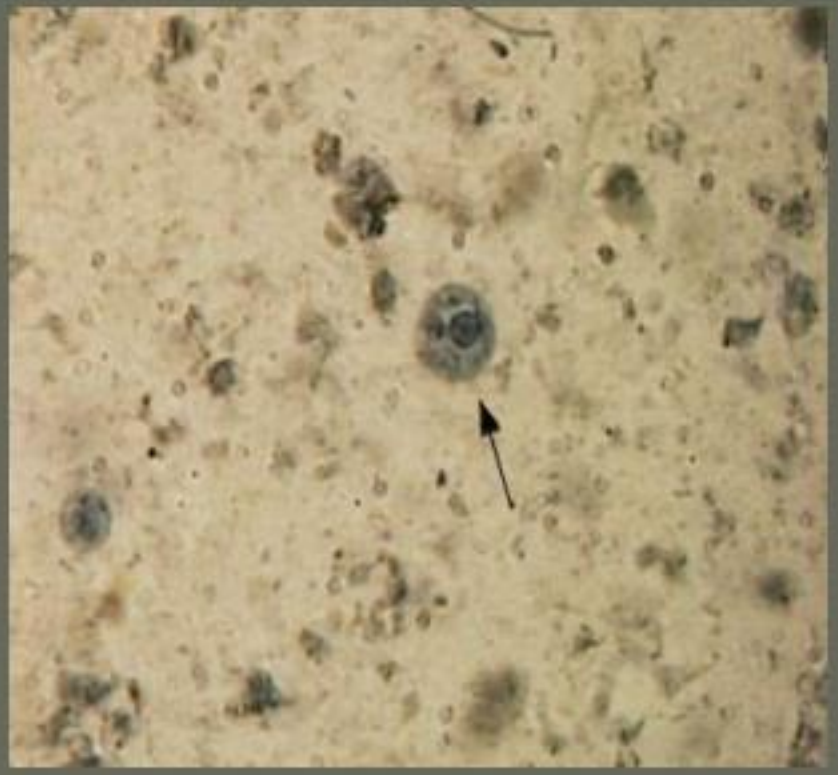
Amebas are unicellular organisms common in the environment: many are parasites of vertebrates and invertebrates. Relatively few species inhabit the human intestine and only ***Entamoeba histolytica*** is identified as a human intestinal pathogen.

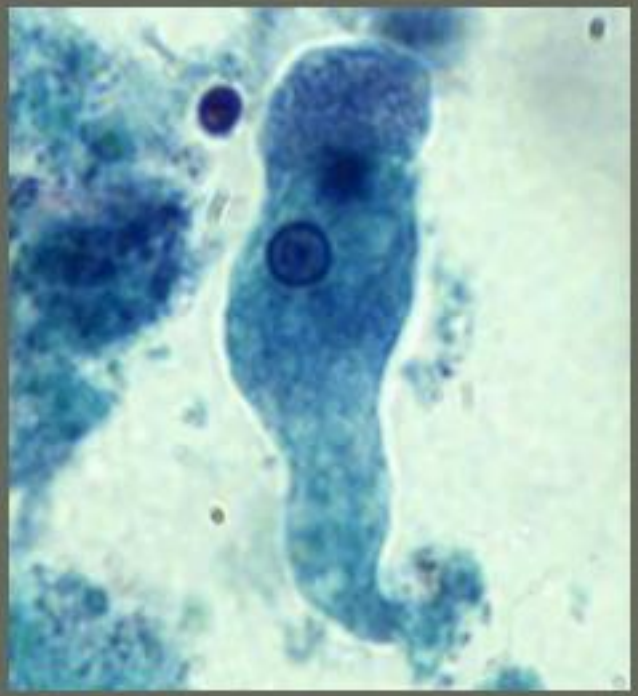
Entamoeba histolytica is an anaerobic parasitic protozoan, part of the genus

Entamoeba. ***E. histolytica*** is estimated to infect about **50 million people worldwide.**

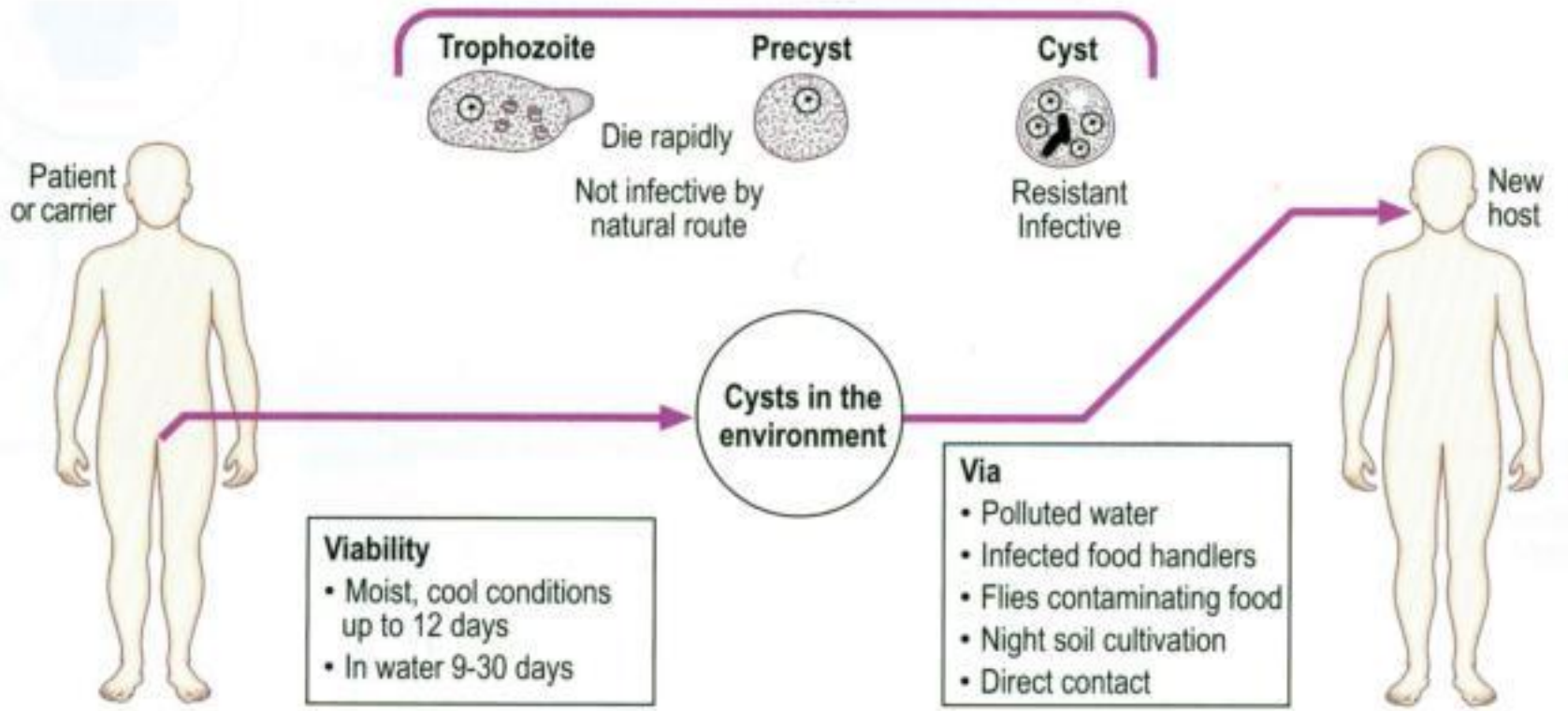
Many older textbooks state that **10%** of the world population is infected.

Cyst & Trophozoit

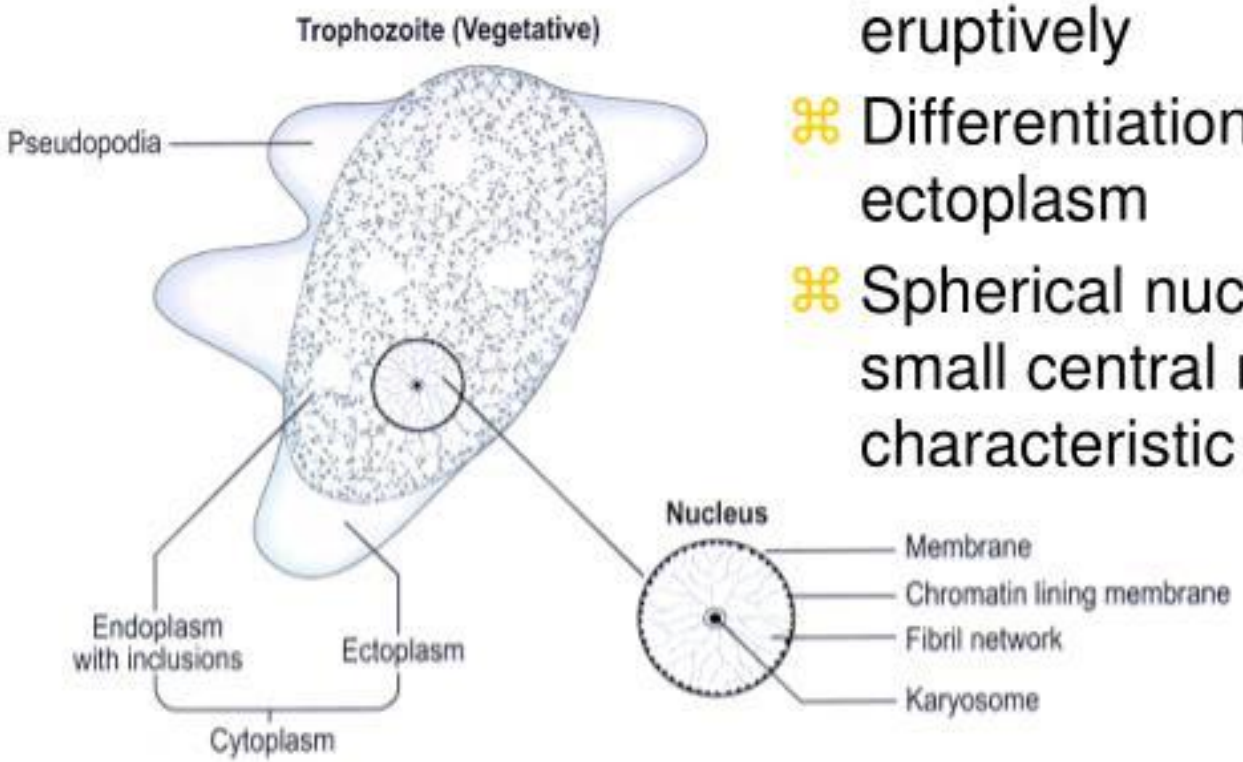




trophozoites and cysts

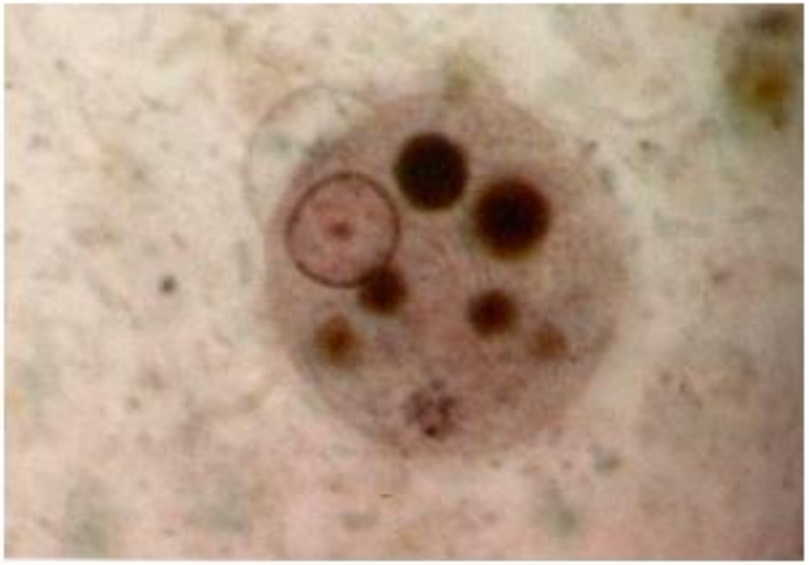


trophozoites and cysts



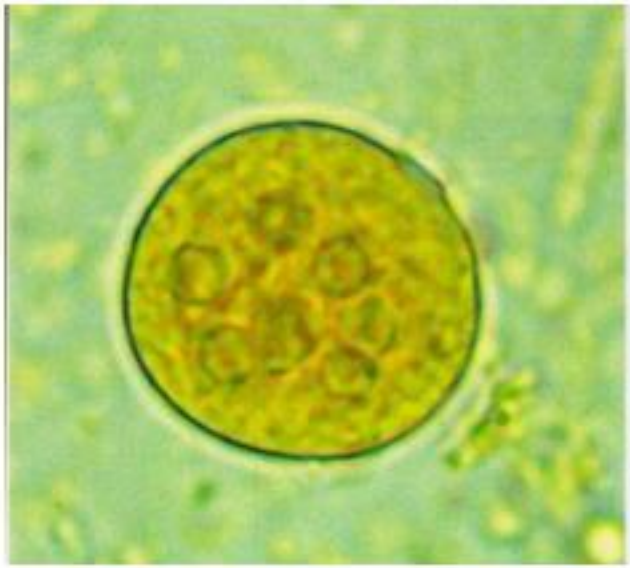
- multiple well defined pseudopodia often extended eruptively
- Differentiation into endo- and ectoplasm
- Spherical nucleus (4-7 μm) with small central nucleolus and characteristic radial spokes

trophozoites and cysts

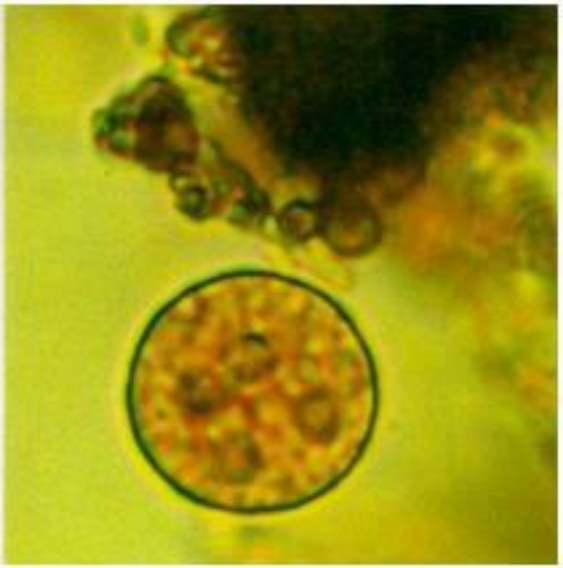


- Trophozoites 20-40 μm diameter
- Ribosomes arranged in helical patterns
- Tissue forms often contain phagocytosed RBCs

Entamoeba cysts (light microscopy)



E. coli

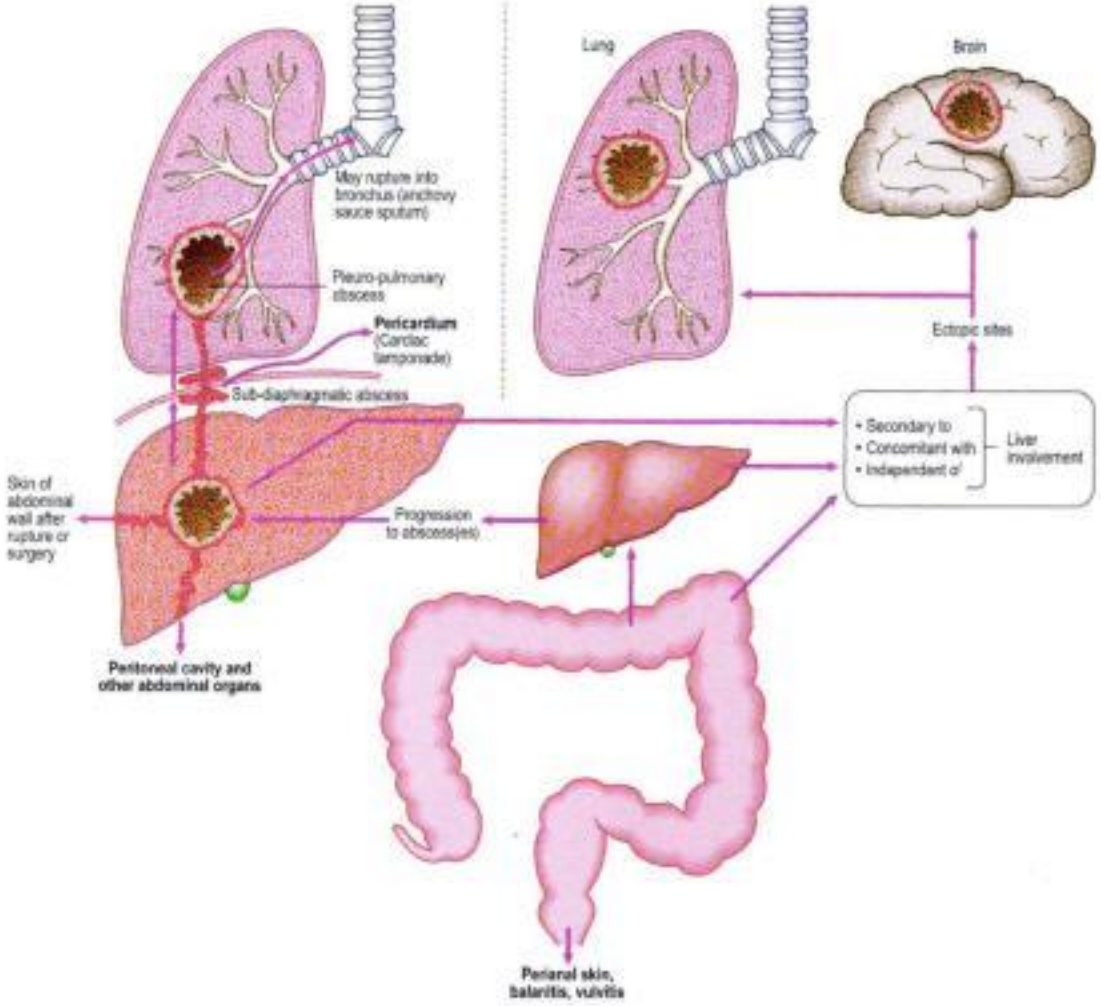


E. histolytica

Colitis is the most common form of disease associated with amoebae

- Gradual onset of abdominal pain, watery stools containing mucus and blood
- Some patients have only intermittent diarrhea alternating with constipation
- Fever is uncommon
- Formation of ulcers

Extraintestinal amebiasis



Genus Plasmodium

- Consists of 4 species:
  1. P. vivax
  2. P. falciparum
  3. P. malariae
  4. P. ovale

Transmission & Life Cycle

Definitive host	Female Anopheles mosquito
Intermediate host	Man
Infective form	Sporozoites
Portal of entry	Skin
Mode of transmission	Bite of an infected mosquito
Site of localization	First in liver cells & then in RBCs

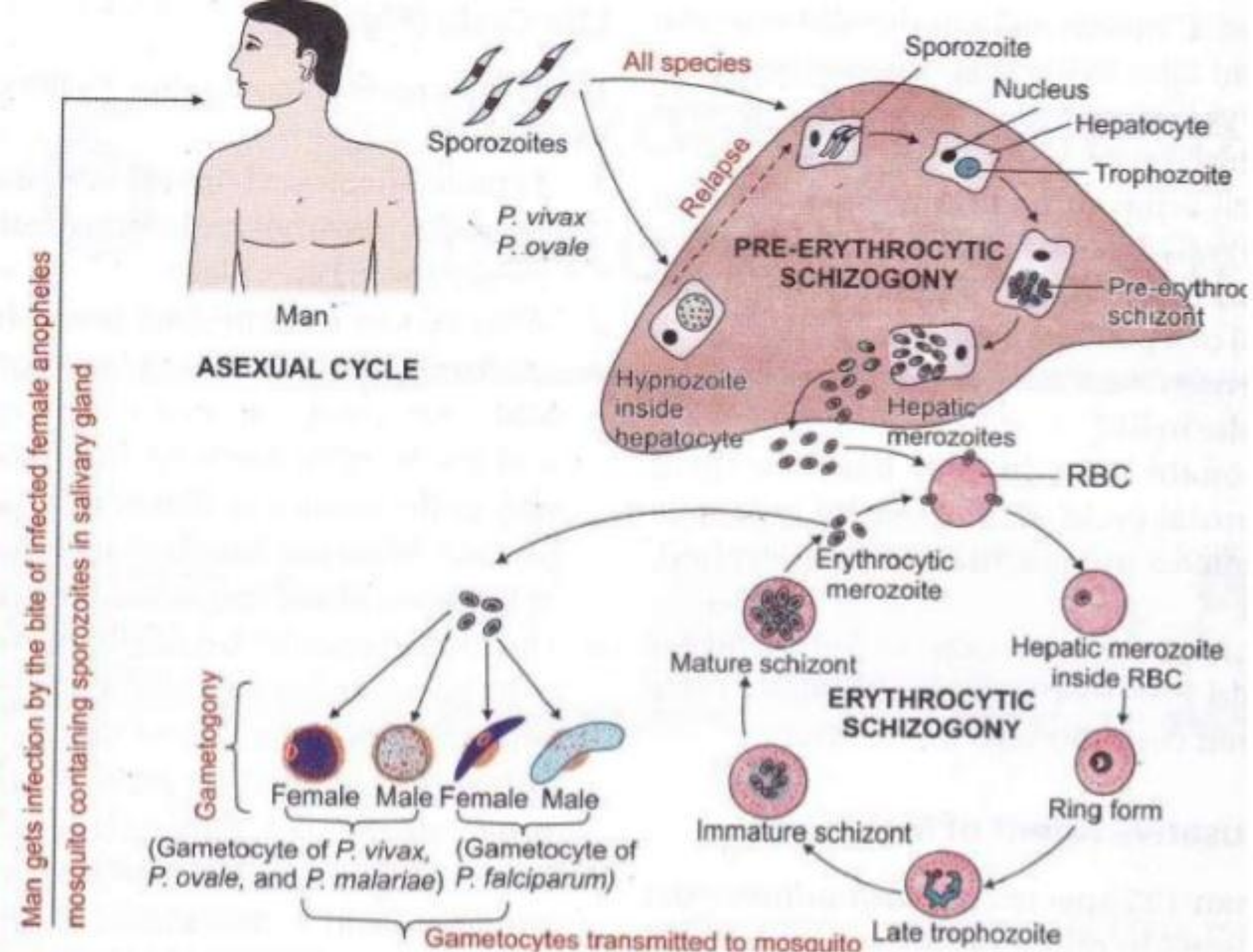
Morphological forms seen in Humans

- In liver:
  1. Sporozoites
  2. Pre erythrocytic schizonts
  3. Merozoites – infect RBCs

- In RBCs :
  1. Trophozoites – ring form
  2. Schizonts
  3. Merozoites – released by the rupture of schizonts – infect other RBCs
  4. Gametocytes – micro and macro gametocytes

Morphological forms seen in Mosquito

- Further differentiation & development of gametocytes take place in mosquito
  1. Macro gametes (female gametes) – each macro gametocyte develops in to one macro gamete in the mid gut of mosquito
  2. Micro gametes (male gametes) – one micro gametocyte produces 6 to 8 micro gametes by exflagellation.
  3. Zygote – Ookinete – Oocyst – rupture – release of Sporozoites – predilection to salivary glands.


Man gets infection by the bite of infected female anopheles mosquito containing sporozoites in salivary gland
Man
ASEXUAL CYCLE
Sporozoites
All species
P. vivax
P. ovale
Relapse
Sporozoite
Nucleus
Hepatocyte
Trophozoite
PRE-ERYTHROCYTIC SCHIZOGONY
Pre-erythroc schizont
Hypnozoite inside hepatocyte
Hepatic merozoites
RBC
Erythrocytic merozoite
Mature schizont
Hepatic merozoite inside RBC
ERYTHROCYTIC SCHIZOGONY
Ring form
Immature schizont
Late trophozoite
Gametogony
Female Male Female Male
(Gametocyte of P. vivax, P. ovale, and P. malariae)
(Gametocyte of P. falciparum)
Gametocytes transmitted to mosquito

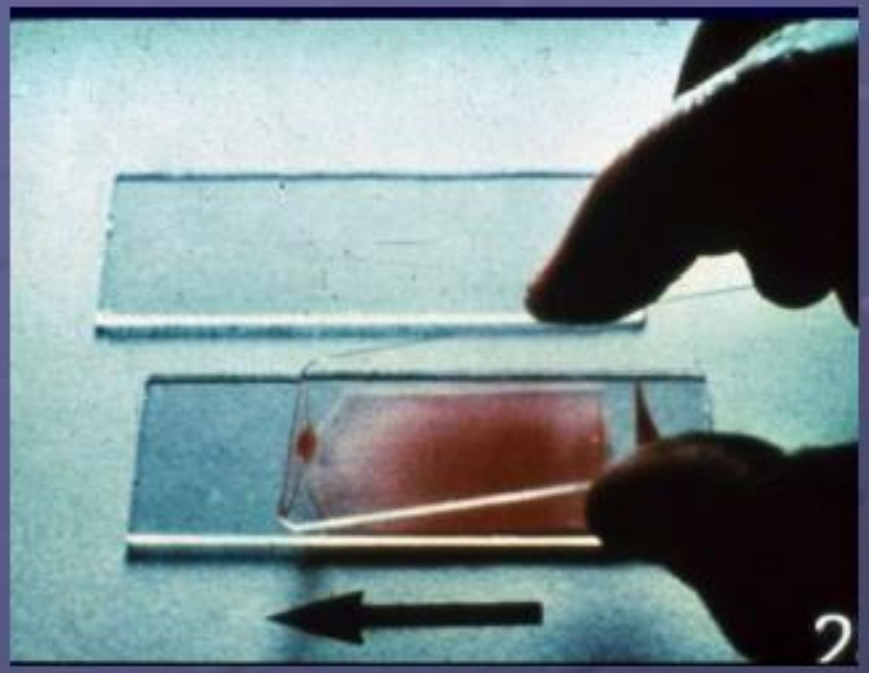
Incubation Period

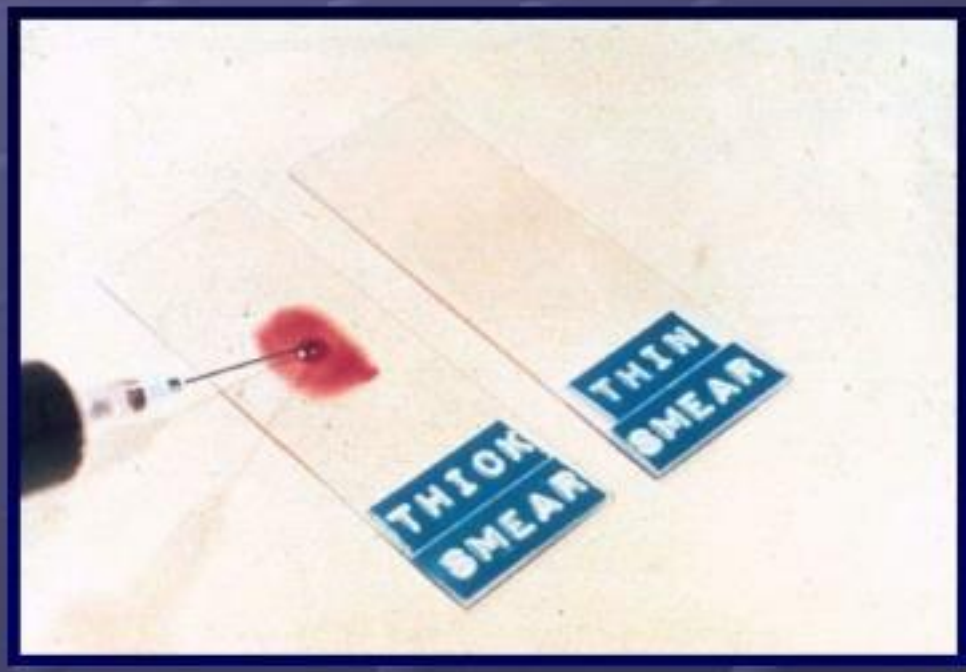
- **Pre-patent Period**
  - Time taken from infection to symptoms
    - *Plasmodium falciparum* 6-12 days
    - *Plasmodium vivax* 10-17 days
    - *Plasmodium ovale* 12-16 days
    - *Plasmodium malariae* 28-30 days

Clinical Features

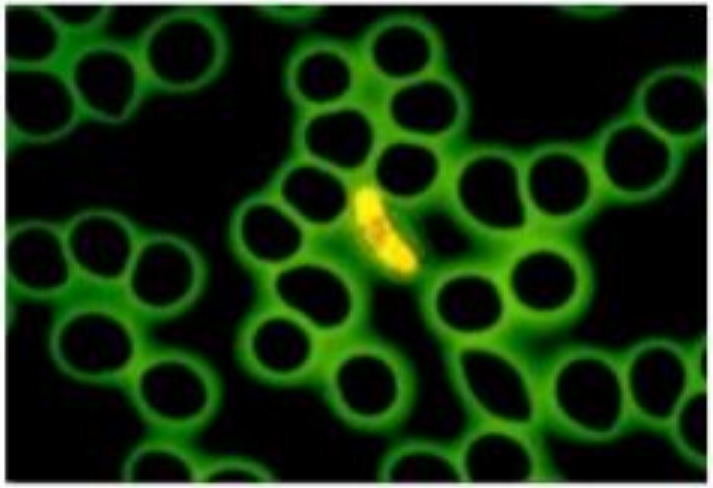
- Anaemia – due to breakdown of RBCs, particularly occurs in falciparum malaria
- Splenomegaly – all forms

Making of Thin & Thick films



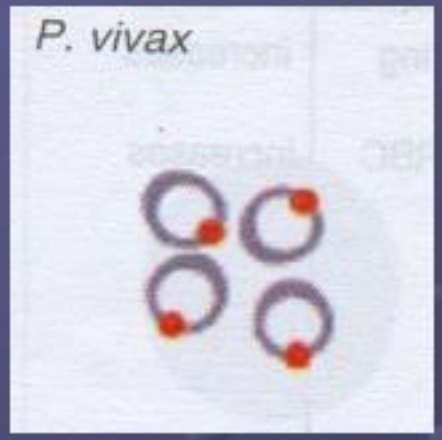


- **FLUORESCENCE MICROSCOPY**
- Kawamoto technique - fluorescent staining method for demonstrating MP
- Blood smear are prepared on slides & are stained with acridine orange & examined under F.Microscope - nuclear DNA is stained green

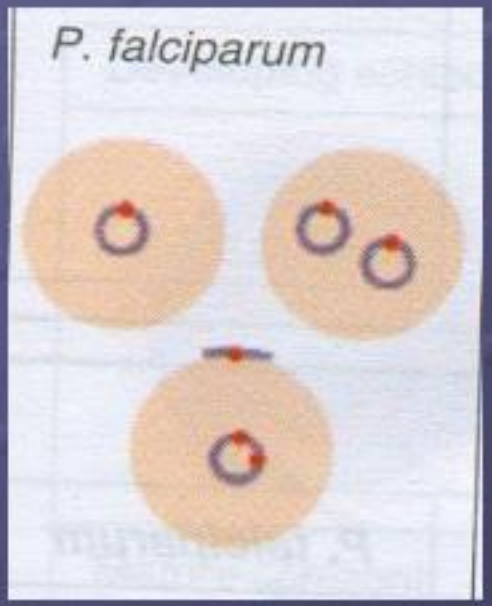


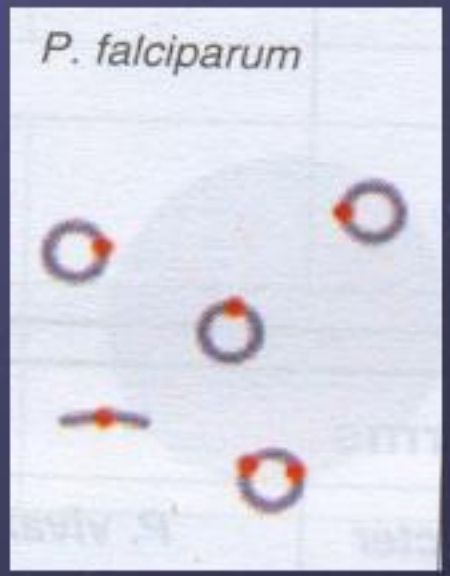
Thin Blood Film

Thick Blood Film

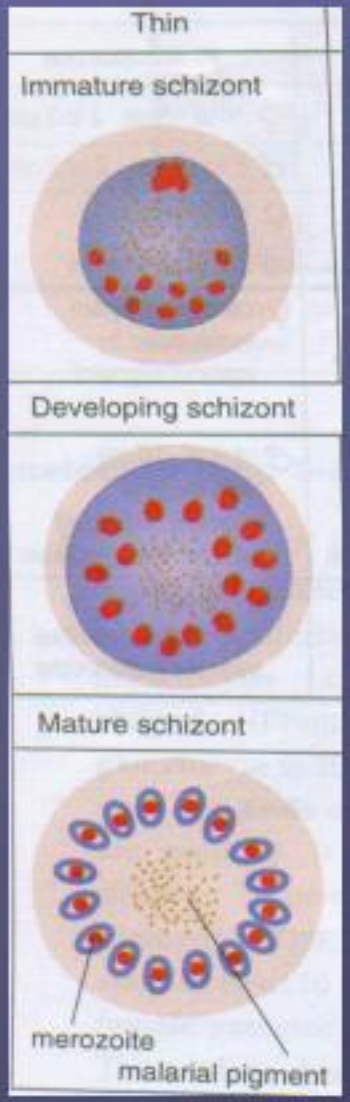


Ring Forms / Trophozoites



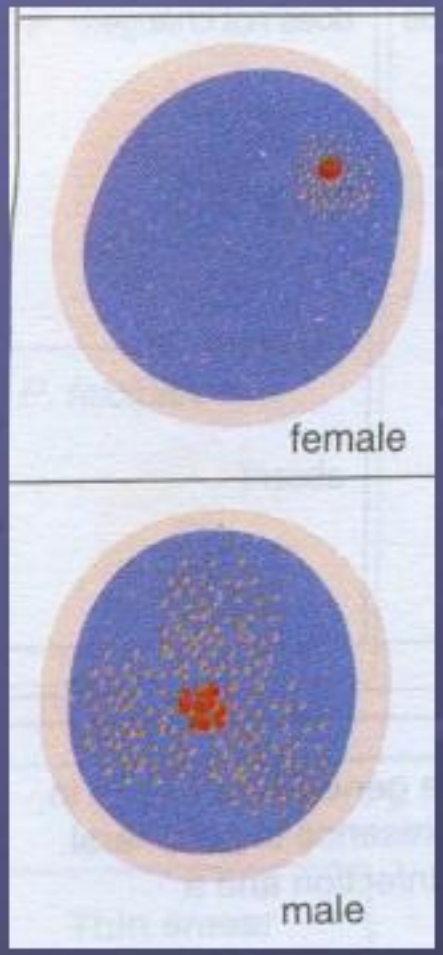


P. vivax

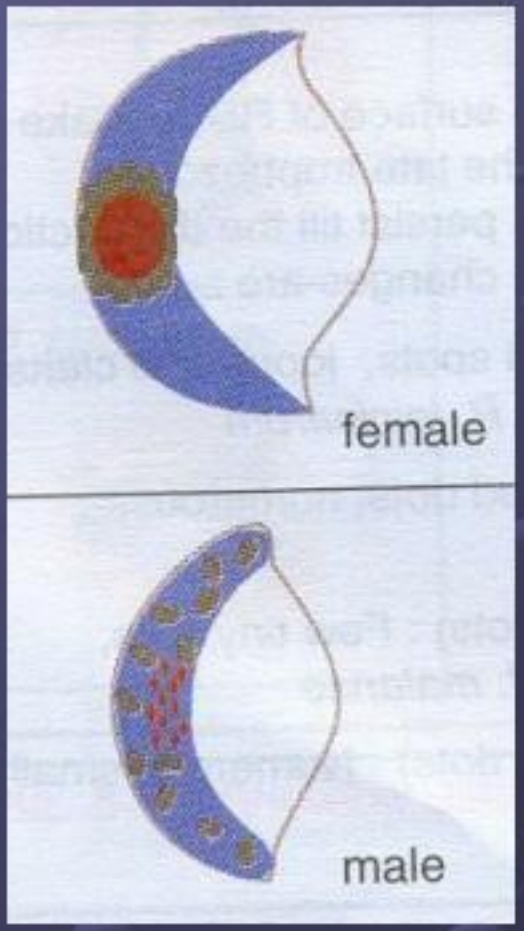


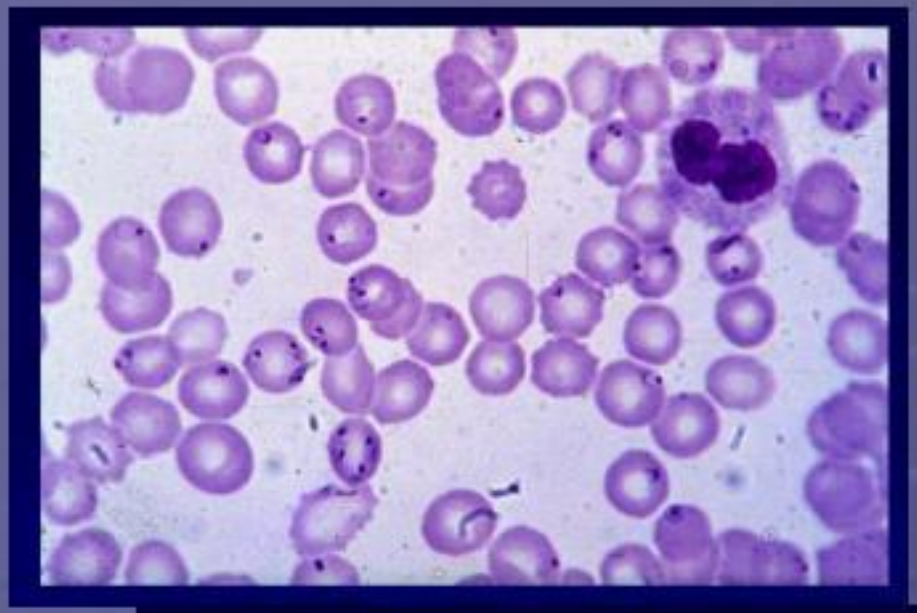
P.falciparum

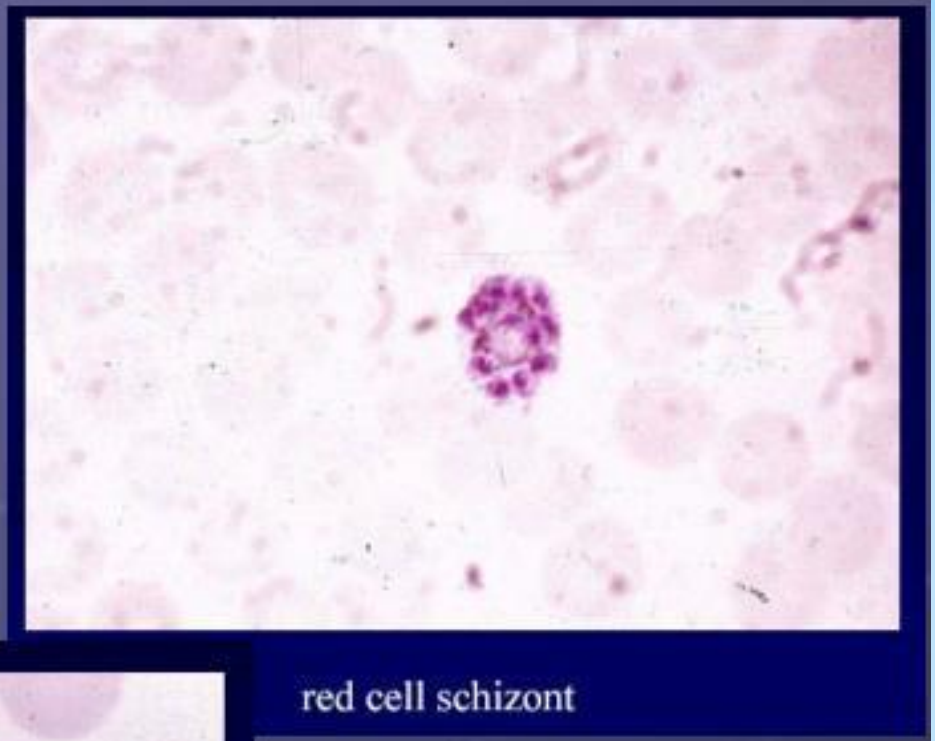
P.vivax



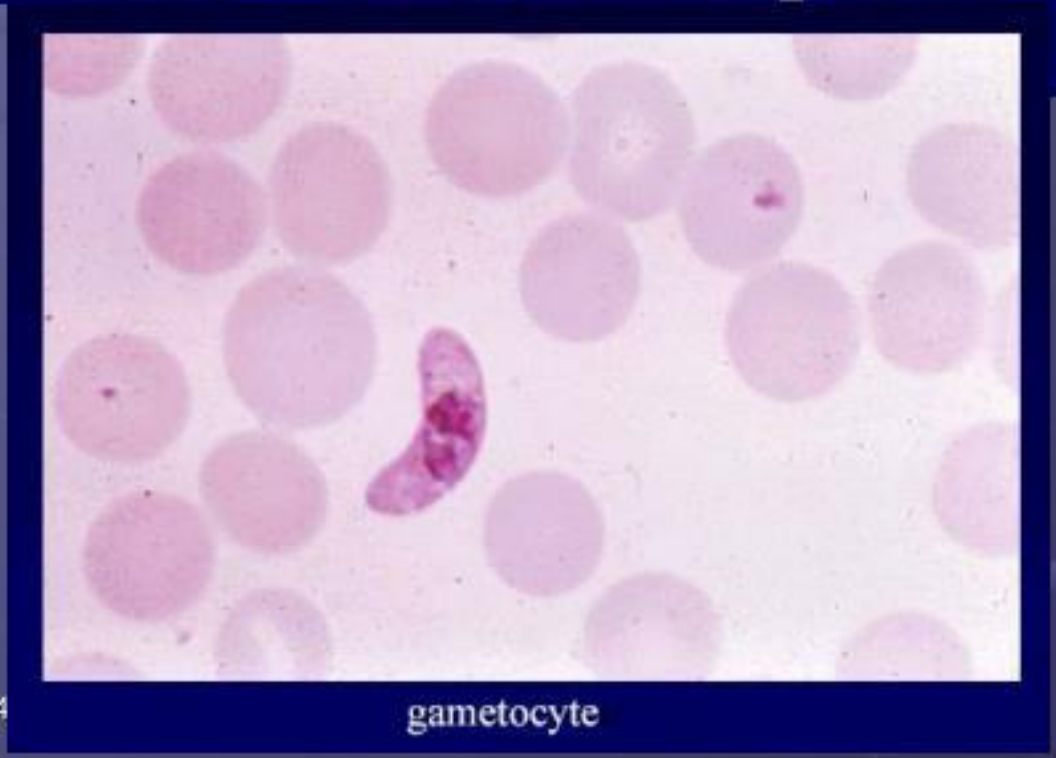
P. falciparum







red cell schizont



gametocyte

TAXONOMIC CLASSIFICATION

D: Eukarya

K: Protista (Alveolata)

P: Apicomplexa

C: Coccidia

O: Eucoccidiorida

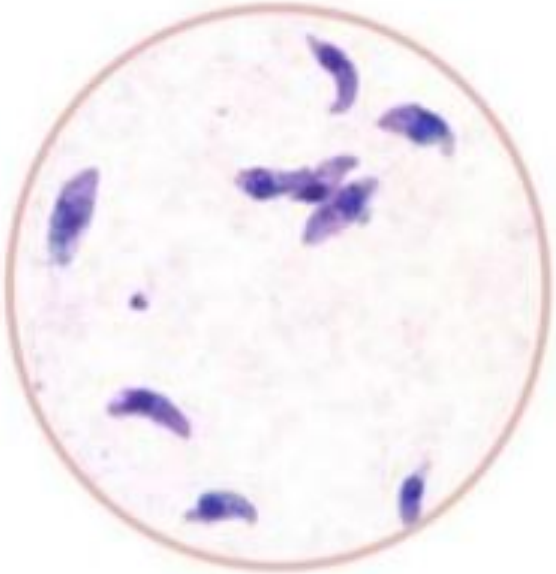
F: Sarcocystidae

G: *Toxoplasma*

S: *gondii*

NEGLECTED PARASITIC INFECTION:

Toxoplasmosis



Toxoplasmosis is the **2nd leading cause of death** from foodborne illness in the United States.

CDC

Learn more: www.cdc.gov/parasites/npi/

TOXOPLASMOSIS

- Widely-distributed zoonosis caused by *T. gondii* protozoa.
- The definitive host is the domestic cat and other felines.
- Humans and other mammals are intermediate hosts.

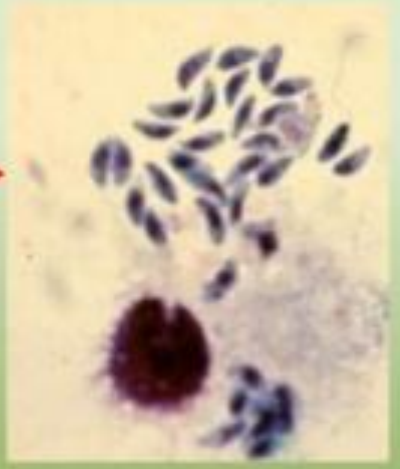
Nicolle and Manceaux (1909)

Toxoplasma gondii exists in four forms

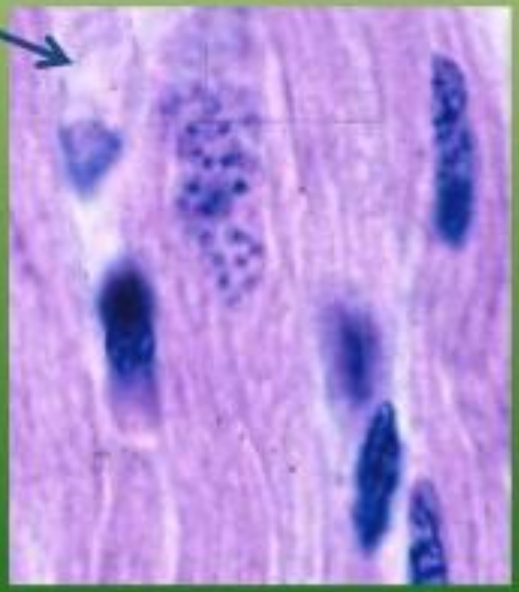
All parasite stages are infectious.

1. **TACHYZOITES**
2. **TISSUE CYSTS**
3. **BRADYZOIT**
4. **OOCYSTS**

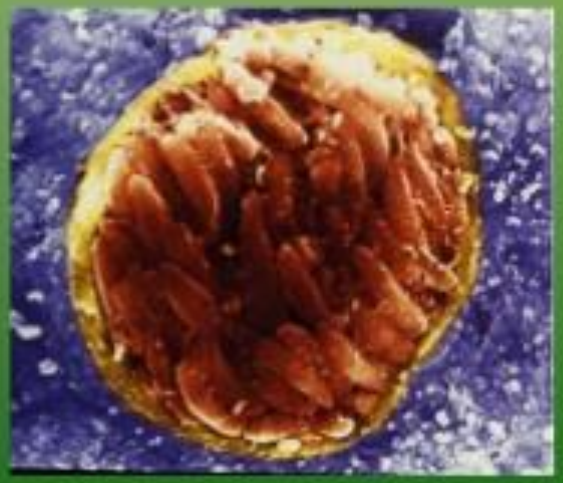
Tachyzoites



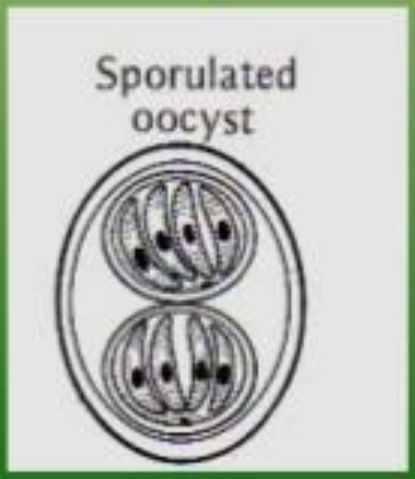
TISSUE CYSTS



Bradyzoites



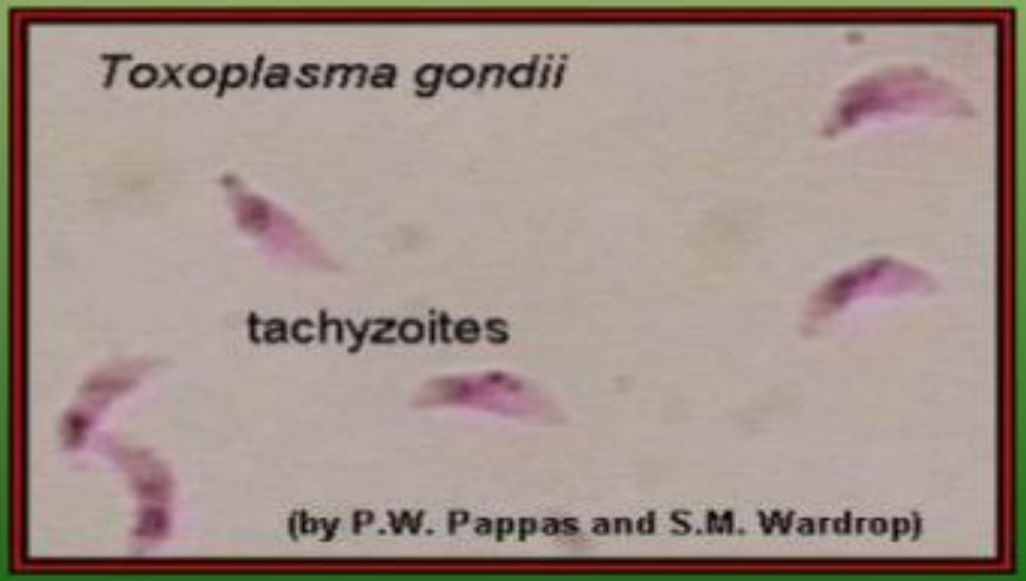
Oocysts



MORPHOLOGY

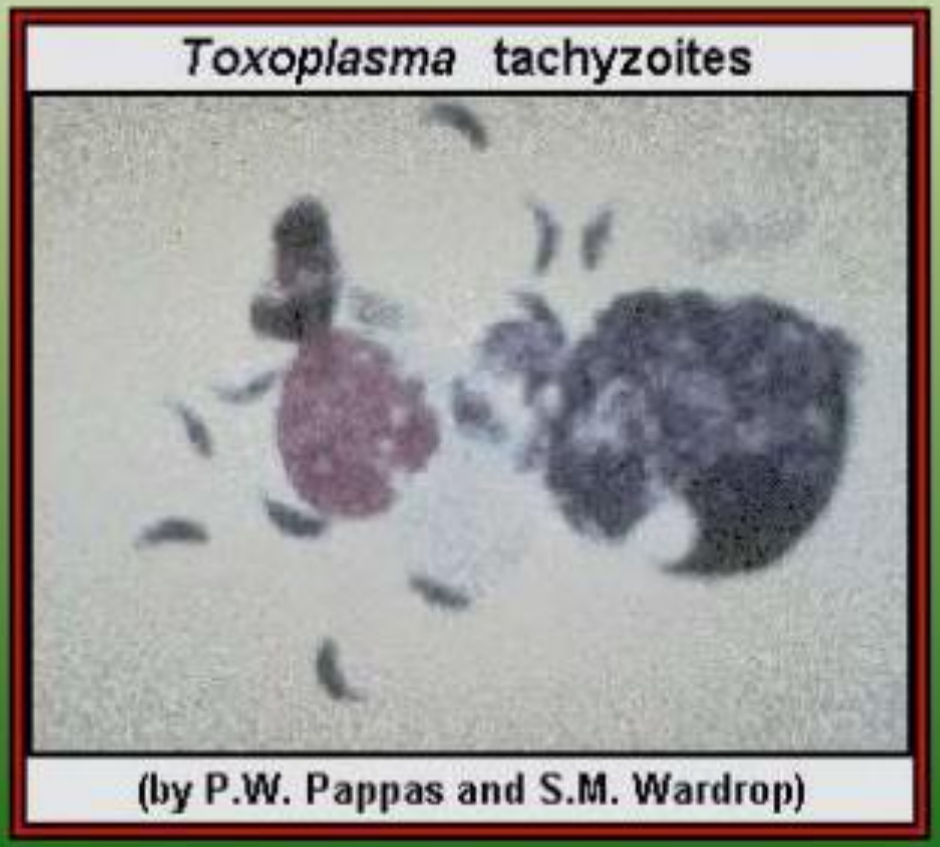
- The intracellular parasites (tachyzoite) are 3x6μ and crescent shaped organisms that are enclosed in a parasite membrane to form a cyst measuring 10-100 μ in size.

- Cysts in cat feces (oocysts) are 10-13 μ in diameter



MORPHOLOGY

- Form of *Toxoplasma gondii* : tachyzoites.

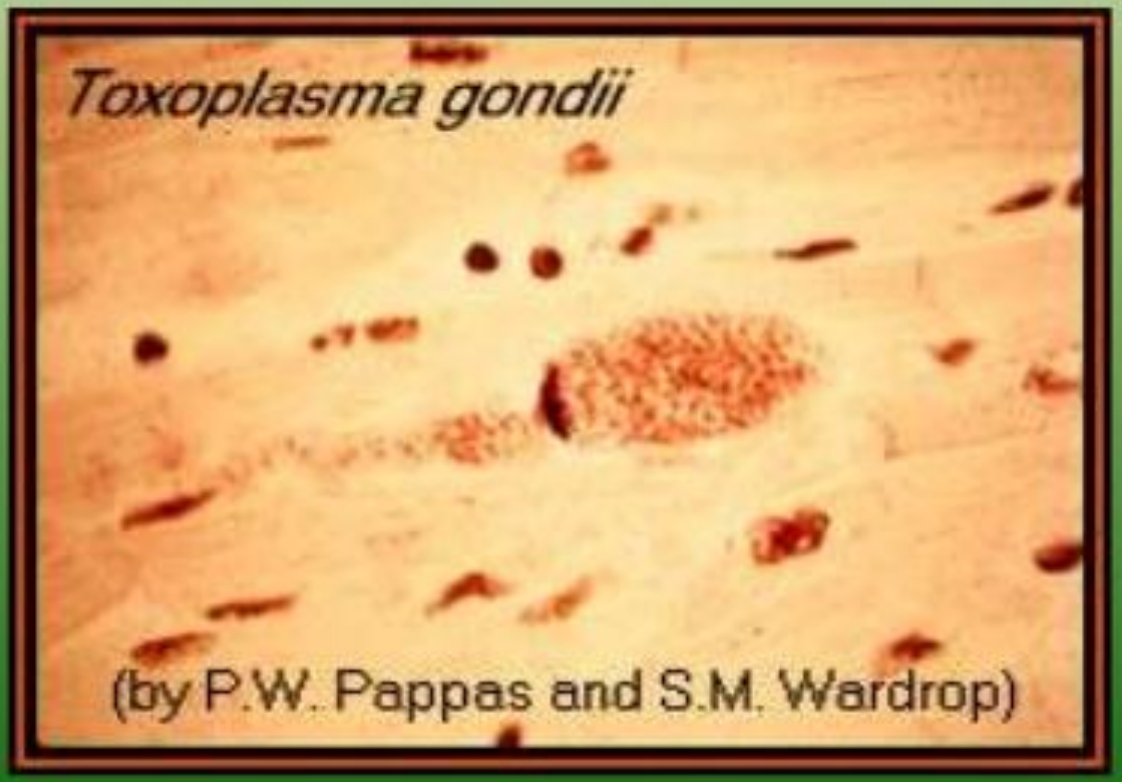


MORPHOLOGY

- Intracellular tachyzoites of *Toxoplasma gondii*.

MORPHOLOGY

- A zoitocyst of *Toxoplasma gondii* filled with bradyzoites; this zoitocyst is in cardiac muscle.



SYMPTOMS

Common symptoms of T. *gondii* infection in cats includes:

- Fever;
- Ocular inflammation;
- Anorexia;
- Lethargy;
- Abdominal discomfort; and
- Neurologic abnormalities (Vollaire).

The Parasite

- **Phylum** — **Sarcomastigophora**
- **Order** — **Kinetoplastida**
- **Family** — **Trypanosomatidae**
- **Genus** — **Leishmania**

Leishmania Parasites and Diseases

SPECIES	Disease
*Leishmania tropica** *Leishmania major** *Leishmania aethiopica* *Leishmania mexicana*	Cutaneous leishmaniasis
Leishmania braziliensis	Mucocutaneous leishmaniasis
*Leishmania donovani** *Leishmania infantum** *Leishmania chagasi*	Visceral leishmaniasis

* Endemic in Saudi Arabia

Morphology

- **Promastigote**
- **Amastigote**

Flagella

Kinetoplast

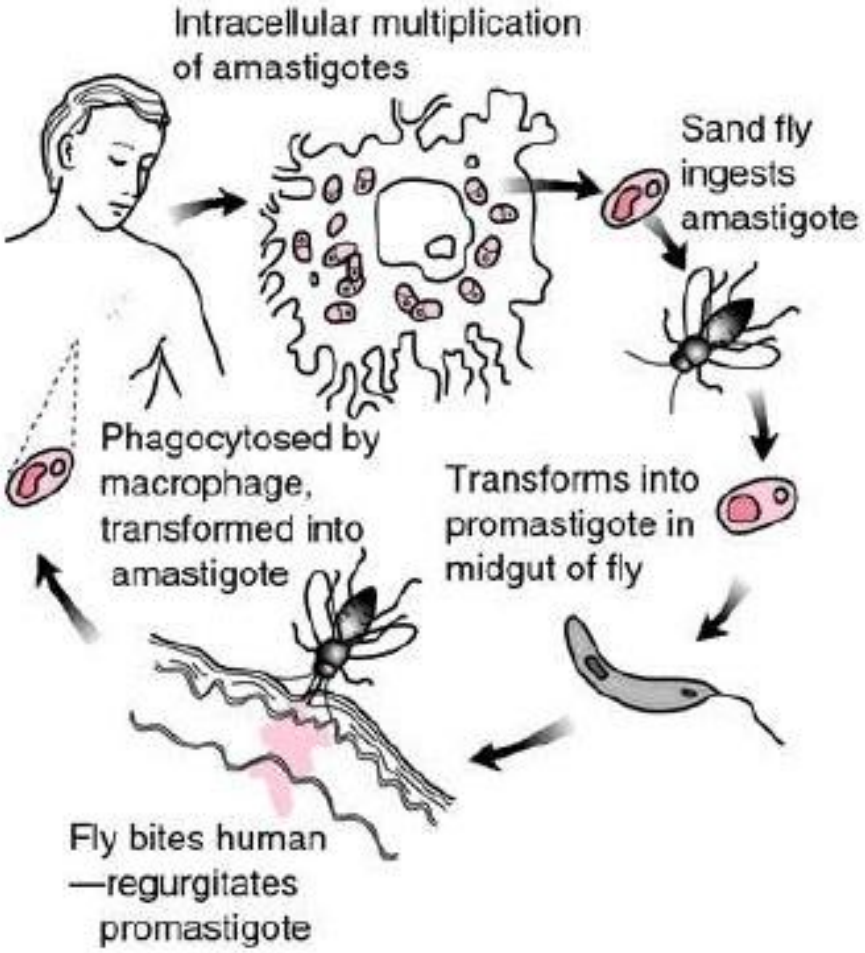
Golgi

Nucleus

Cytoskeleton

Morphology and Life Cycle

- Amastigotes measure 2-3 micrometers, with a large nucleus and Kinetoplast.
- Amastigotes mainly live within cells of the RE system, but have been found in nearly every tissue and fluid of the body.

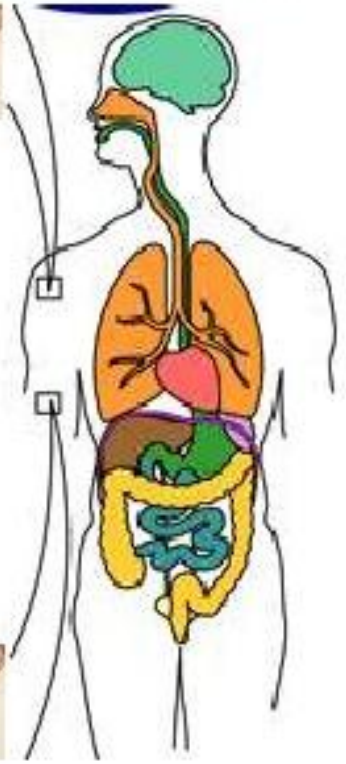


Life cycle

- The organism is transmitted by the bite of several species of blood-feeding **sand flies** (Phlebotomus) which carries the Promastigote in the anterior gut and pharynx. It gains access to mononuclear phagocytes where it transform into Amastigote and divides until the infected cell ruptures.

1- Sand-fly bites animal and ingests blood infected with *Leishmania*

2- Sandfly bites human and injects *Leishmania* into skin



3- Another sandfly bites human and ingests blood infected with *Leishmania*

4- Cycle continues when sandfly bites another human or animal reservoir

Clinical types of cutaneous leishmaniasis

- **Leishmania major:** Zoonotic cutaneous leishmaniasis: wet lesions with severe reaction
- ***Leishmania tropica*:** Anthropologic cutaneous leishmaniasis: Dry lesions with minimal ulceration

Oriental sore (most common) classical self-limited ulcer

Cutaneous leishmaniasis

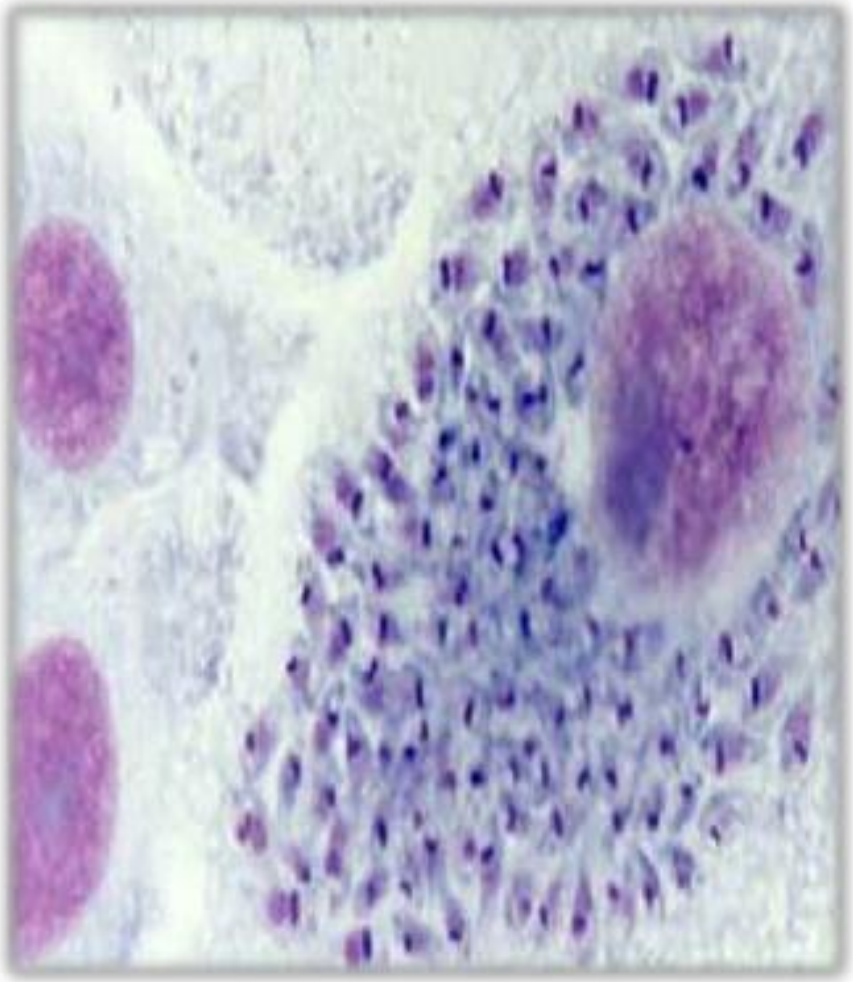
Diffuse cutaneous leishmaniasis

Leishmaniasis recidiva

Cutaneous leishmaniasis

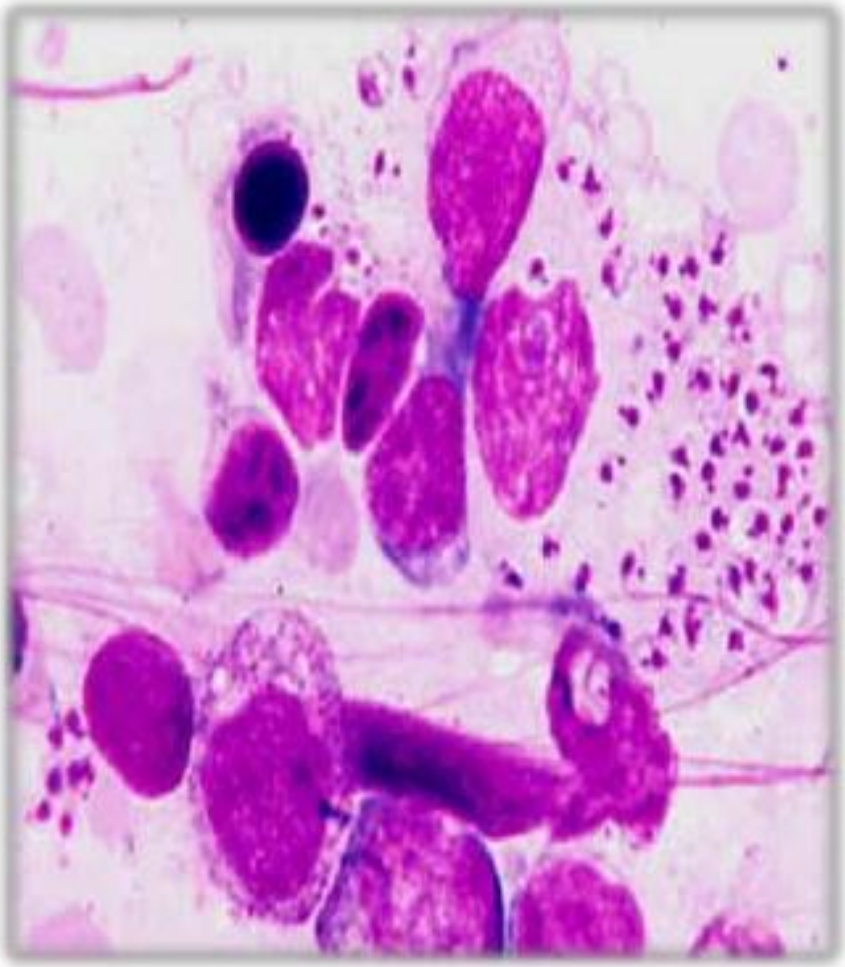
Diagnosis:

- Smear: Giemsa stain – microscopy for LD bodies (Amastigote)
- Biopsy: microscopy for LD bodies or culture in NNN medium for promastigotes



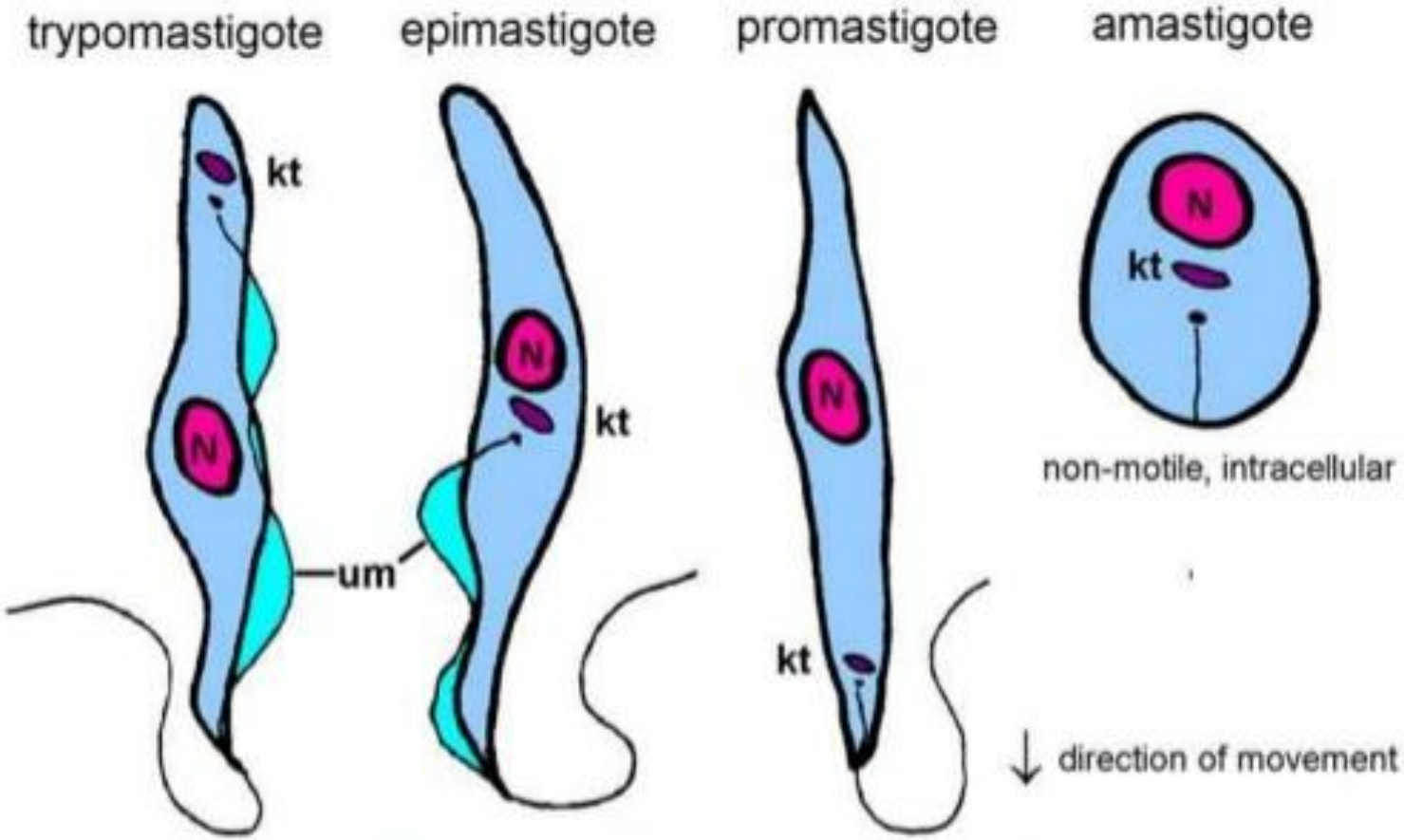
L. donovani bodies

- L. donovani bodies may be demonstrated in buffy coat preparations of blood and bone marrow aspirate. Aspirates taken from enlarged lymph nodes show parasites in 60 percent of cases.



Trypanosomatidae

Stages:



Trypomastigote:
blood stream form;
infective form
replicative

Epimastigote:
replicative stage
in insect

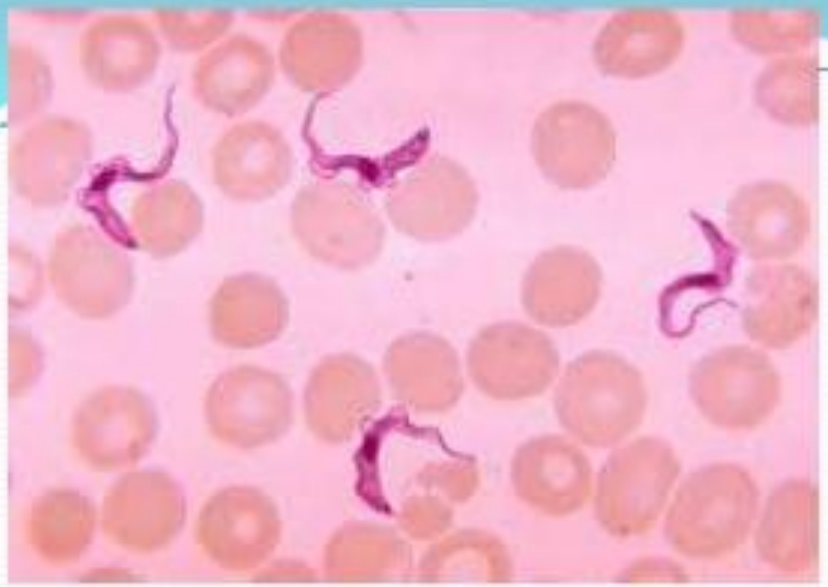
Promastigote:
infective stage of
Leishmania spp.

Amastigote:
non-motile;
intracellular,

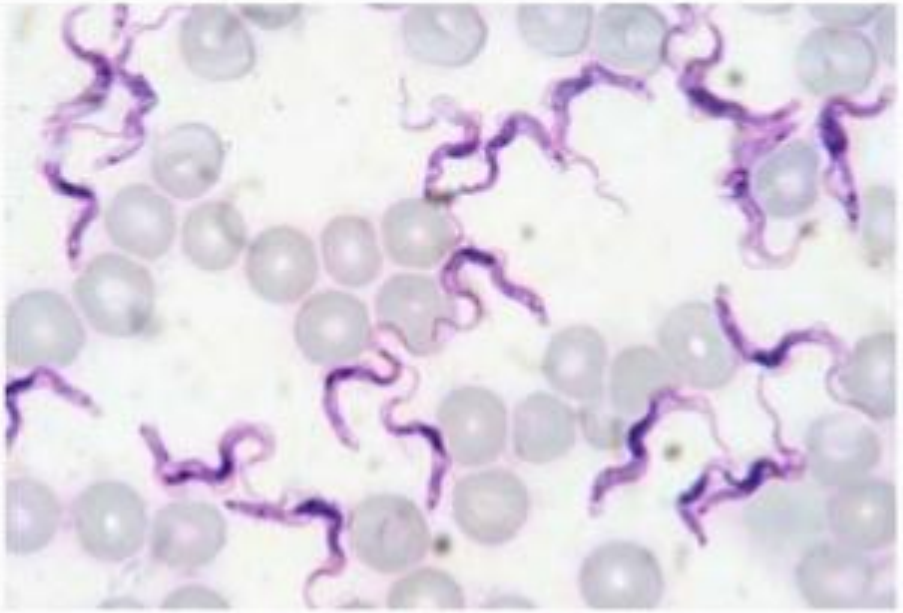
stage in vertebrate

CLASSIFICATION

- *Trypanosoma brucei*- African sleeping sickness.
- *Trypanosoma cruzi*- Chagas' disease.
- *Trypanosoma rangeli*- found in blood of man in Venezuela and also in Colombia.



Blood smear

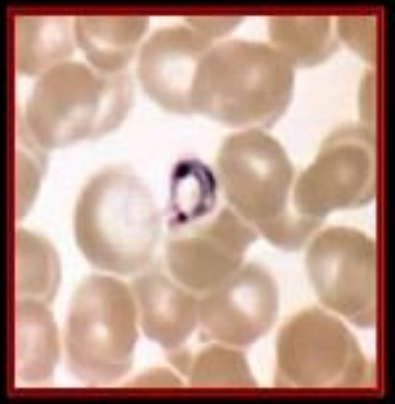


Giemsa stain

TRYPANOSOMA CRUZI AND CHAGAS' DISEASE

- The etiological agent of Chagas' disease is the intracellular protozoan parasite *Trypanosoma cruzi (T. cruzi)*, which is transmitted by the insect vector *Triatoma infestans* (reduviid bug)
- Reduviid bugs live in mud filled walls of huts in rural areas
- The bug bites human hosts and transmits the parasite

Triatoma infestans
(Reduviid bug)



Trypanosoma cruzi
with human erythrocytes

THE LIFE CYCLE OF T. CRUZI

- The life cycle of T. cruzi: The vector, reduviid bug, bites and defecates on host. Parasites, in the form of trypomastigotes, are able to enter the blood via mucous membranes or a cut. During cell invasion, the trypomastigotes transform into amastigotes and undergo multiplication. Parasites are then released into the blood stream as Trypomastigotes where they either spread to other tissues or are taken up by the vector to perpetuate the life cycle

CHAGAS DISEASE

- **Acute stage**: Immediate reaction to infection
  - Only occurs in about 1% of people infected
  - Swelling of the eye, tiredness, fever, rash, loss of appetite
  - Can be fatal for infants, young children and immunocompromised recipients
- **Intermediate**: 8 to 10 weeks after infection
  - No symptoms
- **Chronic**: 10 to 20 years after infection
  - Enlarged heart and digestive tract
  - Can result in heart failure
- **Little effective therapy (toxic drugs/low cure rates)**

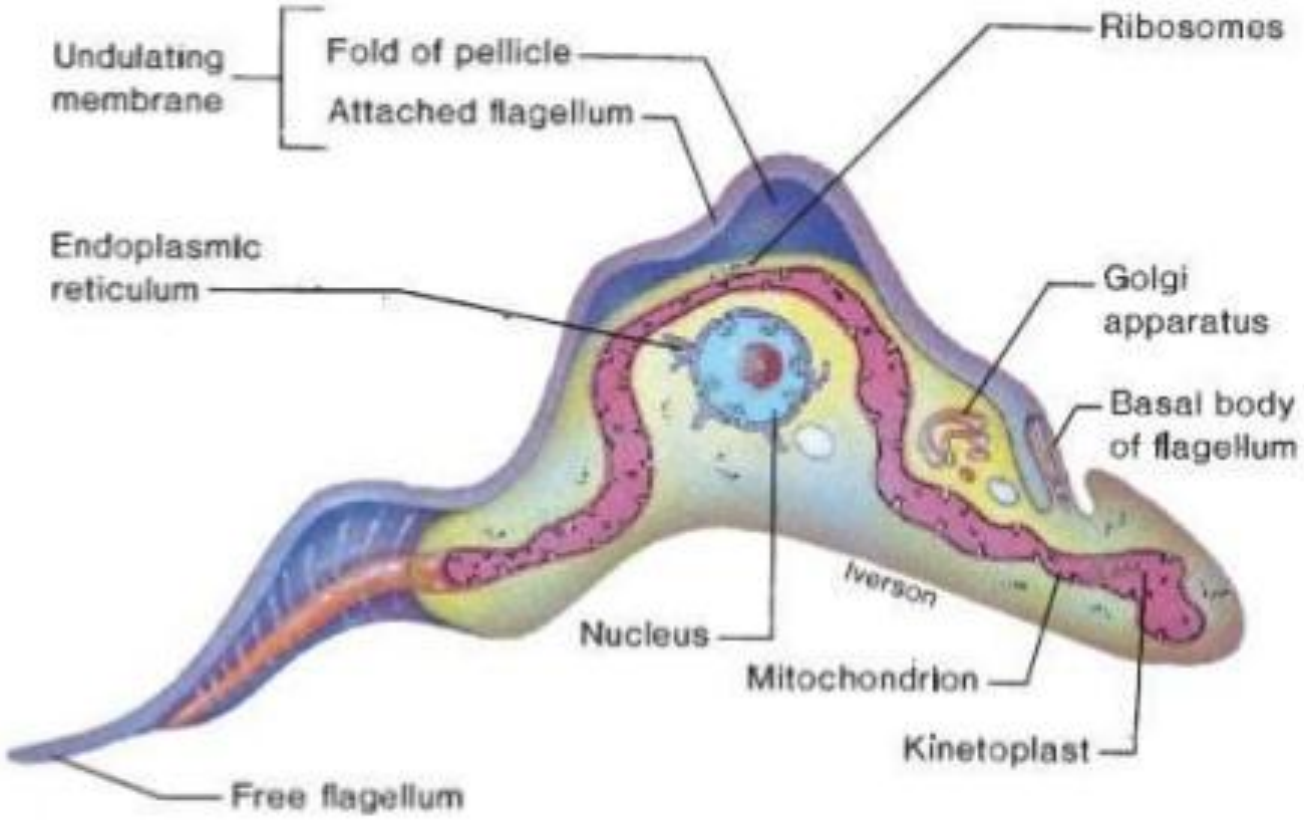
PROGRESS OF THE DISEASE

- Many people may remain asymptomatic for life and never develop Chagas-related symptoms. However, an estimated 20 - 30% of infected people will develop debilitating and sometimes life-threatening medical problems over the course of their lives.

DIAGNOSIS

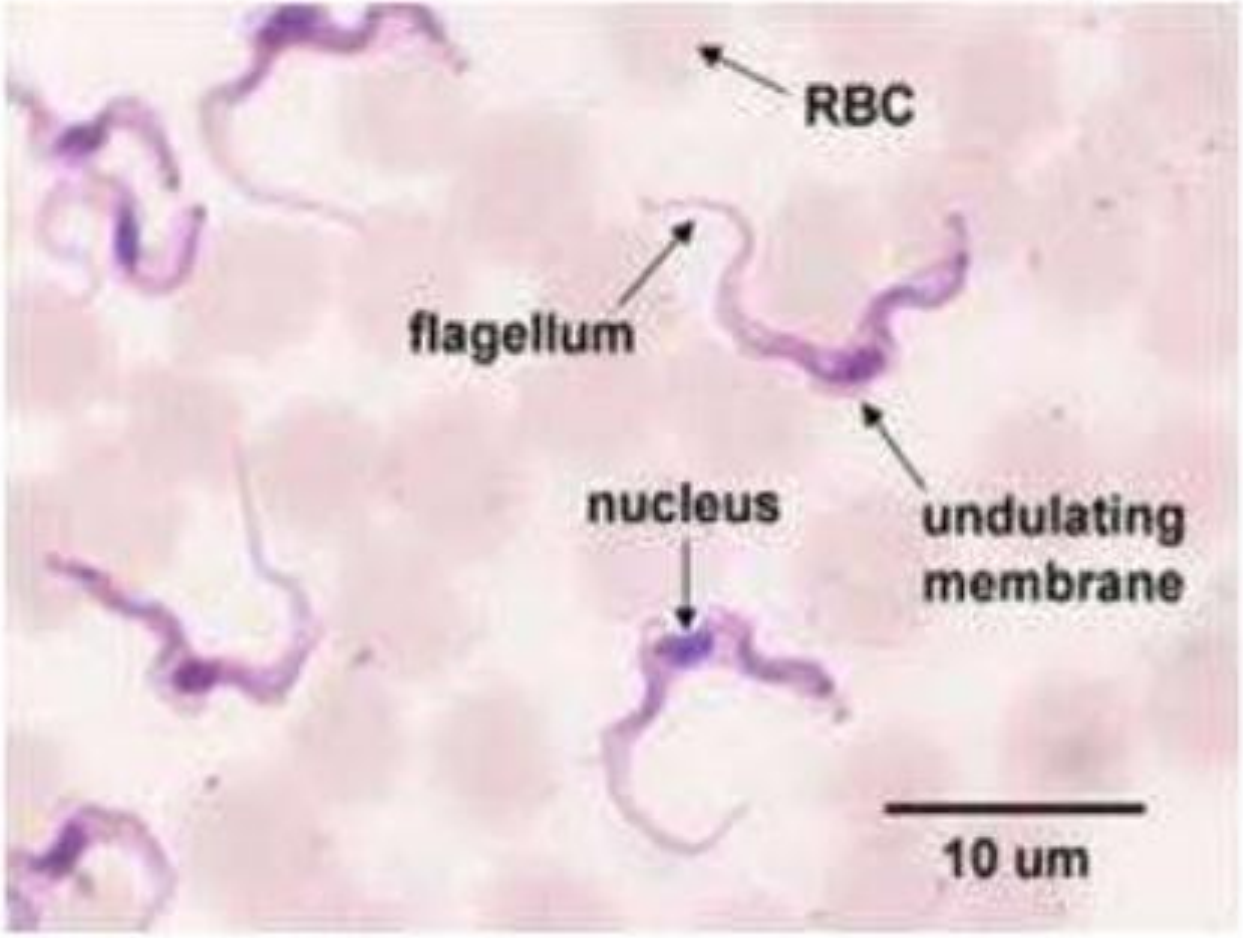
- The diagnosis of Chagas disease can be made by observation of the parasite in a blood smear by microscopic examination. A thick and thin blood smear are made and stained for visualization of parasites.

Trypanosoma brucei Morphology



Trypanosoma brucei

- stained under bright-field microscope:



Trypanosoma brucei

- *T. brucei* causes Human African Trypanosomiasis (HAT) or sleeping sickness

- *T. brucei* is not killed by the immune system because it has a glycoprotein (VSG) coating.

Trypanosoma brucei subspecies

- Two subspecies that are morphologically indistinguishable cause distinct disease patterns in humans:

  1) *T. b. gambiense* causes West African trypanosomiasis

  2) *T. b. rhodesiense* causes East African trypanosomiasis

- A third member, *T. b. brucei*, under normal conditions does not infect humans.

Stages of Infection

- In the **first stage**, the trypanosomes multiply in subcutaneous tissues, blood and lymph. This is also called **haemo-lymphatic stage.**

- In the **second stage**, the parasites cross the blood-brain barrier to infect the central nervous system. This is known as the **neurological or meningo-encephalic stage**.

Pathogenesis

- Incubation period: 2 weeks
- Trypanosomal chancre will develop at the site of bite
- Via lymphatics: enlarged lymph nodes especially posterior cervical region (Winterbottom's sign)
- Via blood stream: headache, fever, muscle & joint pain, irregular erythematous rash

Trypanosoma chancre

Winterbottom sign

Coma before death