AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lecture 6.

Pathogenic fungi and protozoa

FACULTY: General Medicine
SUBJECT: Medical microbiology - 2

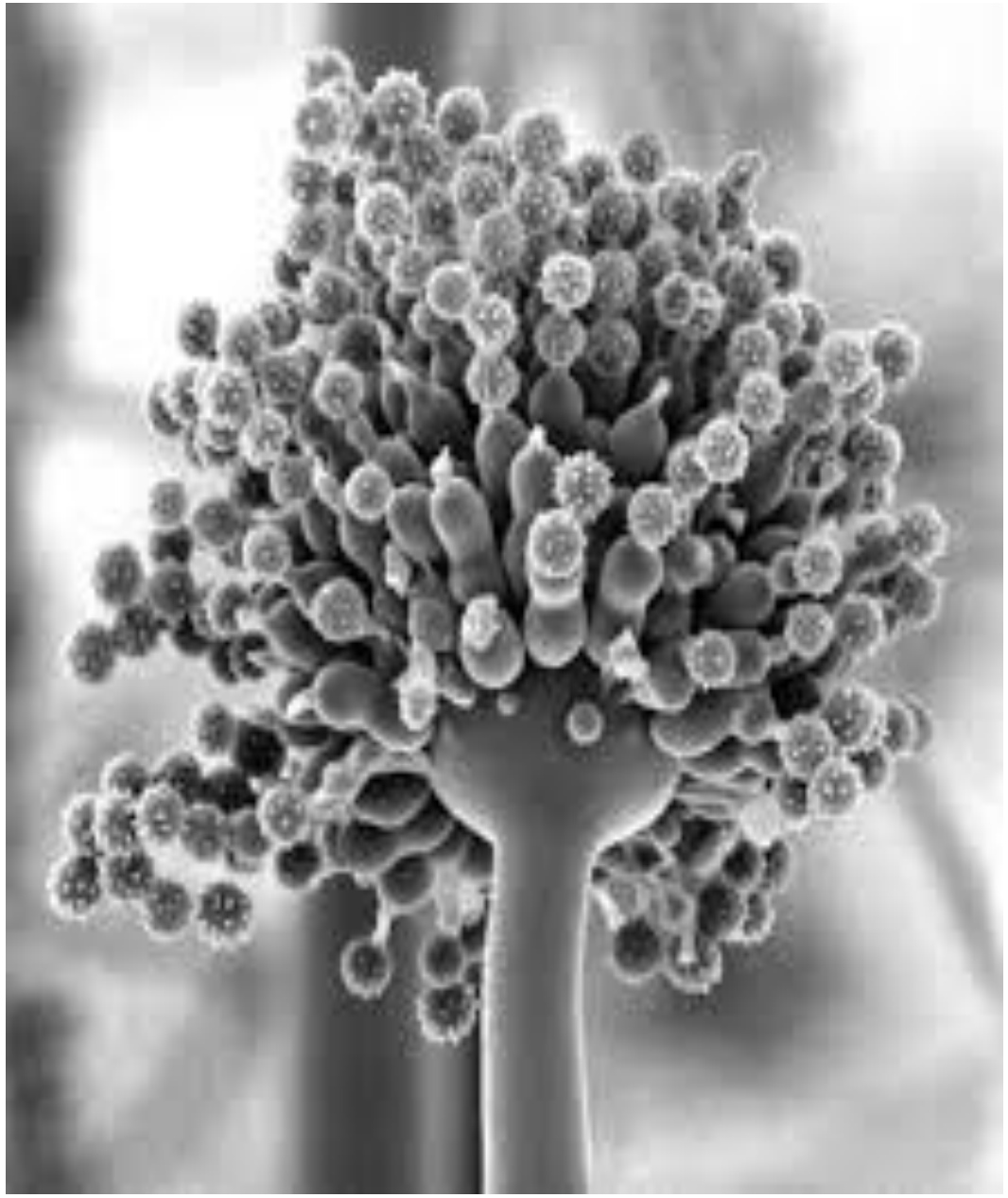
Lecture plan:

1. Classification of fungi that are pathogenic to humans.
2. Classification of mycoses: superficial mycoses (keratomycoses), skin mycoses (dermatomycoses), subcutaneous mycoses (subcutaneous) mycoses, systemic or visceral mycoses and opportunistic mycoses.
3. Morpho-biological features of the causative agents of superficial mycoses (tinea versicolor, black and white piedra), principles of microbiological diagnosis of the diseases they cause
4. Morpho-biological characteristics of the causative agents of skin mycoses (trichophytia, epdermophytia, microsporia, favus), principles of microbiological diagnosis of the diseases they cause
5. Morpho-biological characteristics of the causative agents of subcutaneous mycoses (sporotrichosis, chromomycosis, misetoma), principles of microbiological diagnosis of the diseases they cause
6. Morpho-biological features of the causative agents of systemic (visseral) mycoses (coccidioidosis, histoplasmosis, blastomycosis, paracoccidioidosis), principles of microbiological diagnosis of the diseases they cause
7. Morpho-biological characteristics of the causative agents of opportunistic mycoses (candidiasis, cryptococcosis, aspergillosis, mucoromycosis, pneumocystosis), the principles of microbiological diagnosis of the diseases they cause
8. The concept of mycotoxicosis
9. Causative agents and classification of protozoan infections
10. Morpho-biological characteristics of protozoa belonging to the type Sarcomastigophora (sarcodines and flagellates), principles of microbiological diagnosis of diseases caused by them (amoebiasis, giardiasis, trichomoniasis, leishmaniasis, trypanosomosis)
11. Morpho-biological characteristics of the primitives belonging to the type Apicomplexa (spores), principles of microbiological diagnosis of diseases caused by them (malaria, toxoplasmosis)
12. Morpho-biological characteristics of the protozoa belonging to the type of Ciliophora (ciliated), principles of microbiological diagnosis of the diseases caused by them (balantidiosis)
13. Morpho-biological characteristics of protozoa belonging to the Microspora (invasion) type, principles of microbiological diagnosis of diseases caused by them (microsporidiosis)

Fungi (Fungi, Mycetes, Mycota) **are plant-based, chlorophyll-free, single- or multi-celled eukaryotic organisms**

The branch of microbiology that studies fungi is called mycology

There are pathogenic and non-pathogenic types of fungi

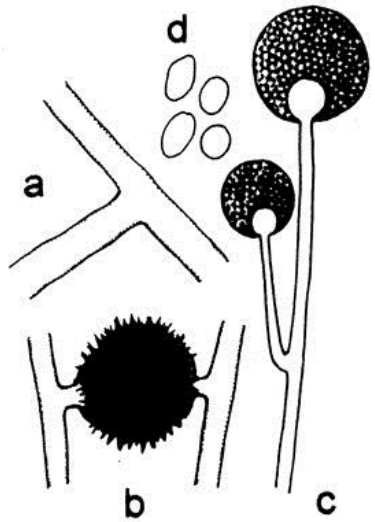


KINGDOM

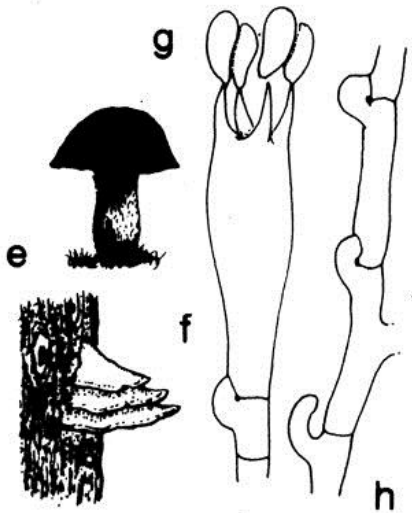
FUNGI

PHYLUM

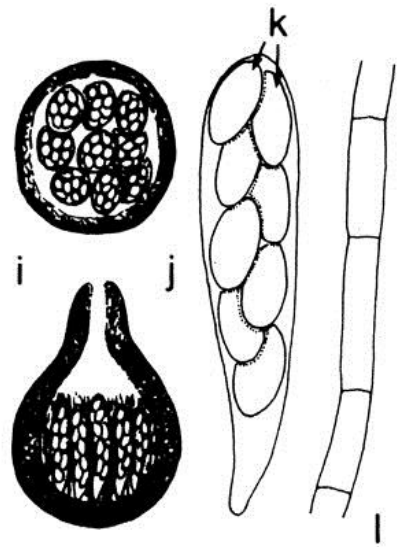
ZYGOMYCOTA



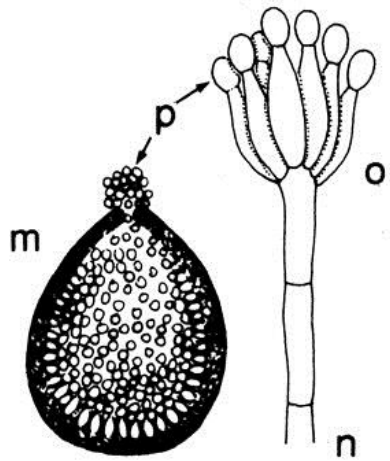
BASIDIOMYCOTA



ASCOMYCOTA



DEUTEROMYCETES (MITOSPORIC FUNGI)



Classification of fungi that are pathogenic for humans:

Zygomycota	***Rhizopus*** ***Absidia*** ***Mucor spp.***
Ascomycota	***Blastomyces*** ***Histoplasma*** ***Microsporium*** ***Trichophyton*** ***Coccidoides*** ***Saccharomyces*** ***Candida spp.***
Basidiomycota	***Cryptococcus neoformans***
Deuteromycota	***Epidermophyton*** ***Paracoccidioides*** ***Sporothrix*** ***Aspergillus*** ***Phialophora*** ***Fonsecaea*** ***Exophiala*** ***Cladophialophora*** ***Bipolaris*** ***Exserohilum***

MYCOLOGY

CLASSIFICATION OF FUNGAL INFECTION

- Superficial
- Cutaneous
- Subcutaneous
- Systemic
- Opportunistic

Mycogenic allergy - allergic conditions and diseases caused by mushrooms or their allergens;

Mycotoxicoses are pathological processes caused by metabolic products of microscopic fungi.

SUPERFICIAL MYCOSES

- 1. Pityriasis (Tinea) versicolor *(Malassezia furfur)*
- 2. Tinea Nigra – *Hortaea/Exophiala werneckii*
- 3. White Piedia – *Trichosporon*
- 4. Black piedra – *Piedraia*

Superficial: Surface mycoses

A. **Superficial mycoses** - infections limited to the outermost layers of the skin and hair. The superficial mycoses are:

Disease	Etiological Agent	Symptoms	Identification of organism
Pityriasis versicolor	*Malassezia furfur*	hypopigmented macules	"**spaghetti and meatballs**" appearance of organism in skin scrapings
Tinea nigra	*Exophiala werneckii*	black macules	black, 2-celled oval yeast in skin scrapings
Black piedra	*Piedraia hortai*	black nodule on hair shaft	black nodule on hair shaft composed of spore sacs and spores
White piedra	*Trichosporum beigelii*	creme-colored nodules on hair shaft	white nodule on hair shaft composed of mycelia that fragment into arthrospores

Malassezia infections

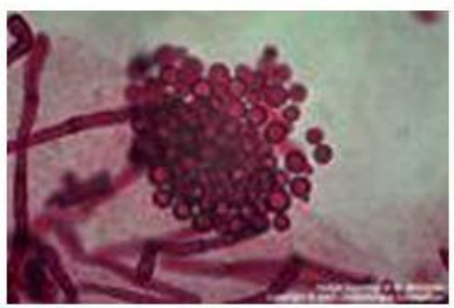
Description:

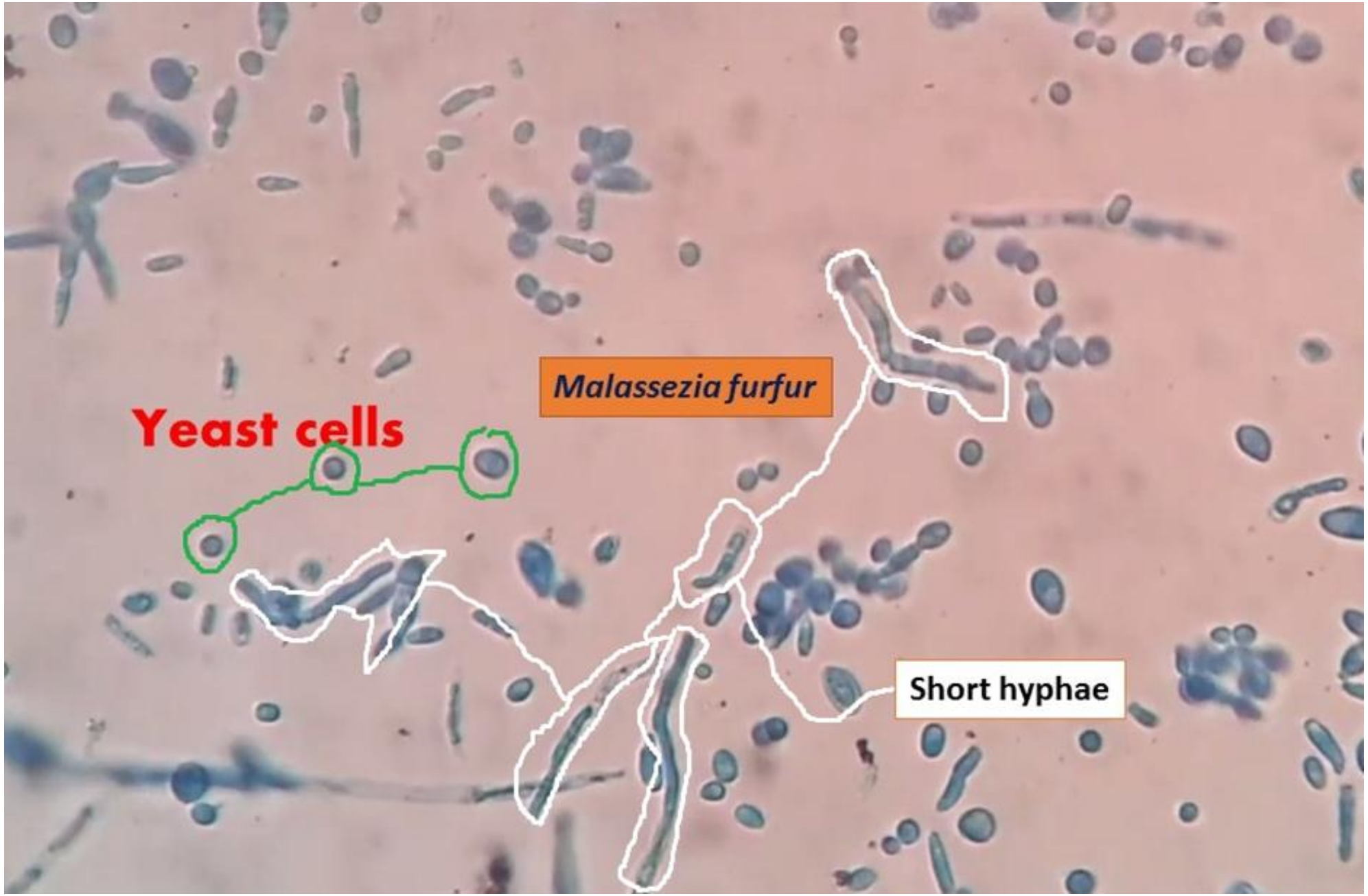
•MALASSEZIA FURFUR (Lipophilic yeast) Saprophytes on skin's lipid secretions

•*Malassezia furfur* is the causative agent of Pityriasis versicolor, Pityriasis folliculitis and it has recently been implicated as a causative agent of seborrhoeic dermatitis and dandruff.

•It has also been recovered in blood cultures from neonate and adult patients undergoing lipid replacement therapy.

•*M. furfur* is a lipophilic yeast living on the skin as part of the normal flora.




Yeast cells
Malassezia furfur
Short hyphae

Pityriasis (Tinea)Versicolor

Fungal infection of the skin

❖This is a very common superficial mycoses caused by a lipophilic yeast *Malassezia* spp. It is seen in all countries but is particularly common in the tropics with more than 60 % of the population infected.

❖The lesions are hypo or hyper pigmented. The etiologic agent is normal flora of man so the disease re-occurs.

❖Multiple patchy lesions (oval shape c fine scales) either light in color or brown

❖Typically occurs on the back, neck, chest, shoulders

General Morphology
Tinea Versicolor

- **Numerous, well-marginated, oval-to-round macules** (change in the color of the skin) **with a <u>fine white scale when scraped.</u>**
- **Pigmentary alteration uniform in each individual.**
  - Red
  - Hypo pigmented
  - Hyperpigmented
- **<u>Scattered over the trunk and neck. Seldom the face.</u>**
- **M. furfur**
  - Normal flora of skin
- **Asymptomatic.**

Superficial mycosis (Pityriasis versicolor)

Superficial mycosis (Pityriasis versicolor)

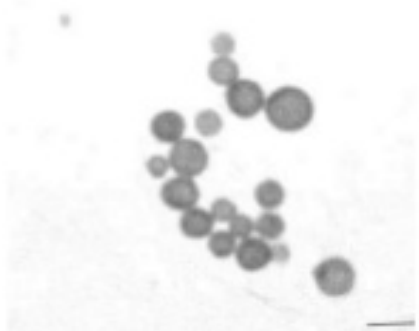



Tinea Versicolor

Malassezia furfur

Definition

- Asymptomatic colonization
- Hypopigmentation
- Hyperpigmentation
- Seborrhoeic dermatitis
- Dandruff
- Fungaemia



Pityriasis versicolor

- **Conditions:**
  - Health
  - Sweat
  - Greasy skin
  - Chronic bacterial infections
  - Steroids

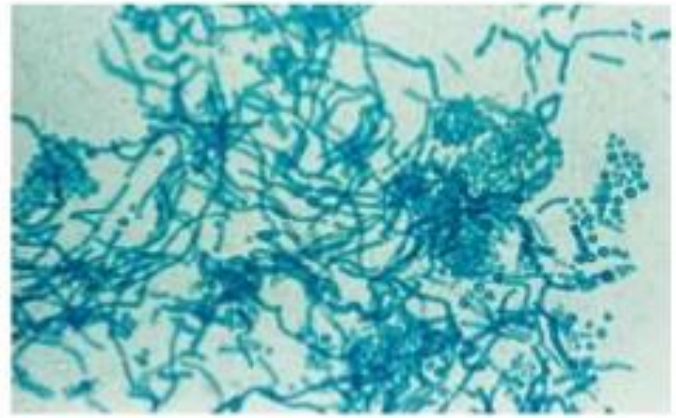
Pityriasis versicolor

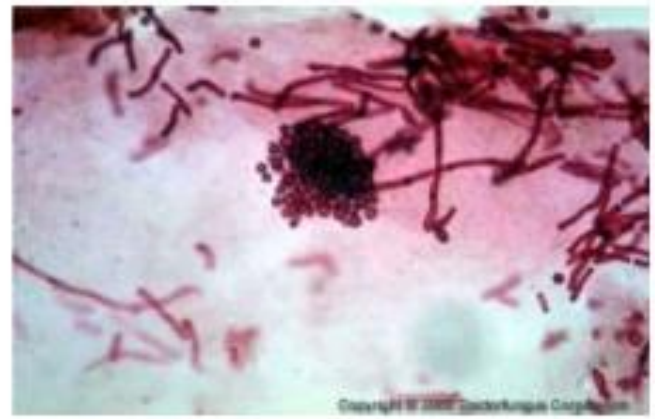
- **Clinical manifestations:**
  - Maculae
  - White, cream, pink, red, brown
  - Scale/signs
  - Painless
  - Not itching

Pityriasis versicolor

Laboratory diagnosis:

- Sampling
  - Scalpel (sharp bladed instrument)
  - Wood ́s lamp (producing ultraviolet radiation)
- Direct
- Culture





Tinea Versicolor

Diagnosis:

- Scrape lightly – fine white scale
- KOH Positive for short hyphae and spores (Spaghetti –hyphae and meatballs-yeasts)
- Woods Light – pale yellow white fluoresce.
- Culture rarely done.

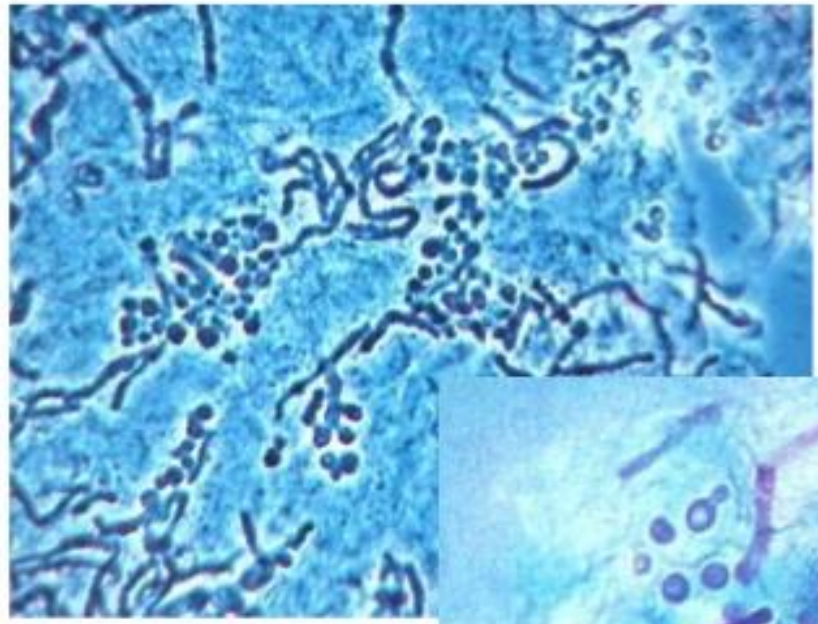
Tinea Versicolor

Direct exam.

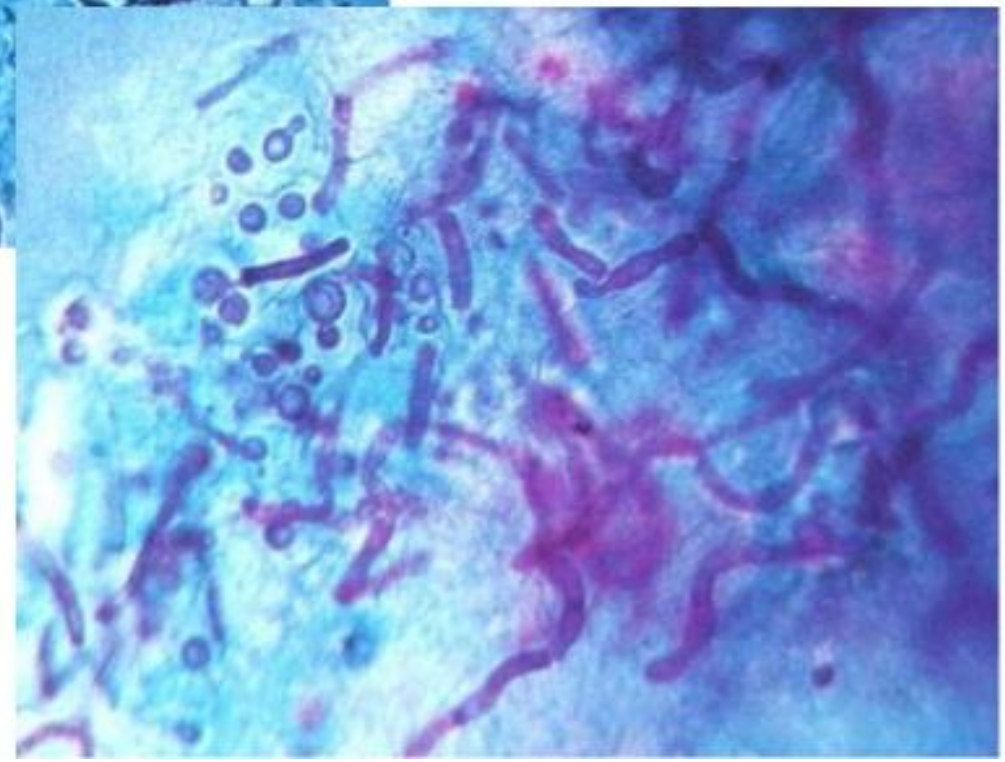
- Skin scraping
- 10% KOH preparation
- Parker Ink

Culture

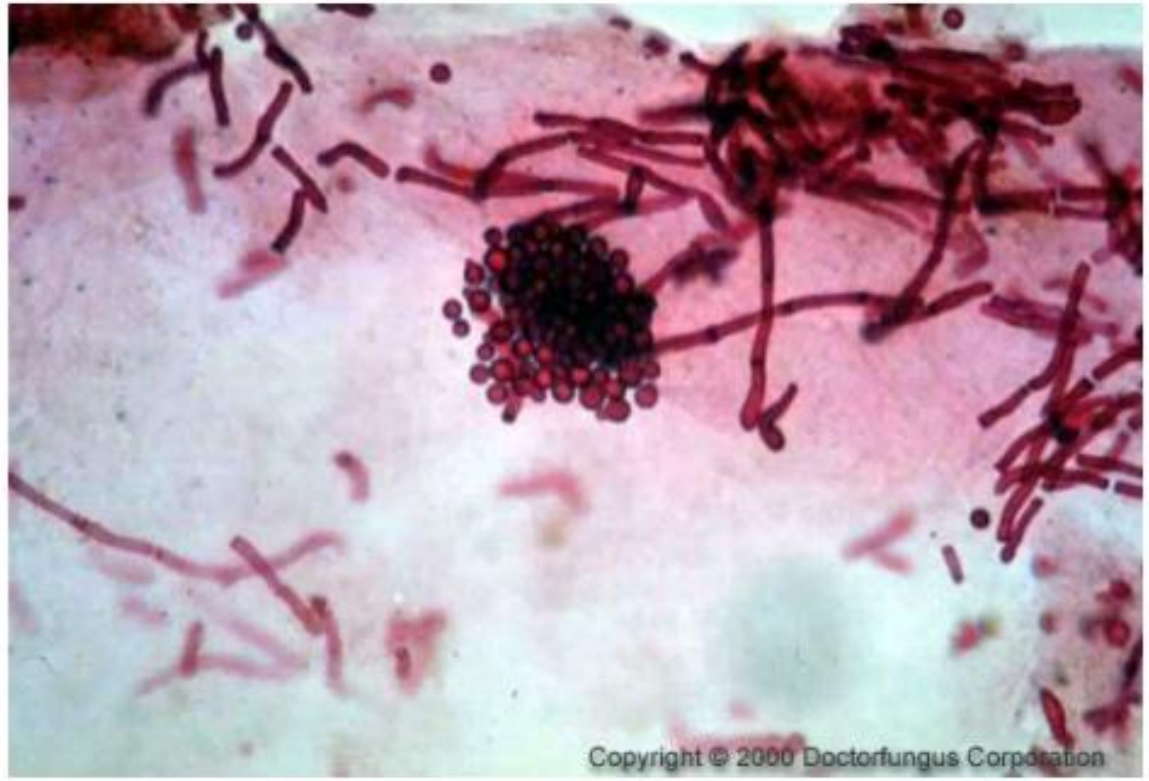
- Sabouraud Dextrose Agar
- Lipophilic yeast
- Flood with Olive oil



- Short fragments of hypha
- cluster of yeast

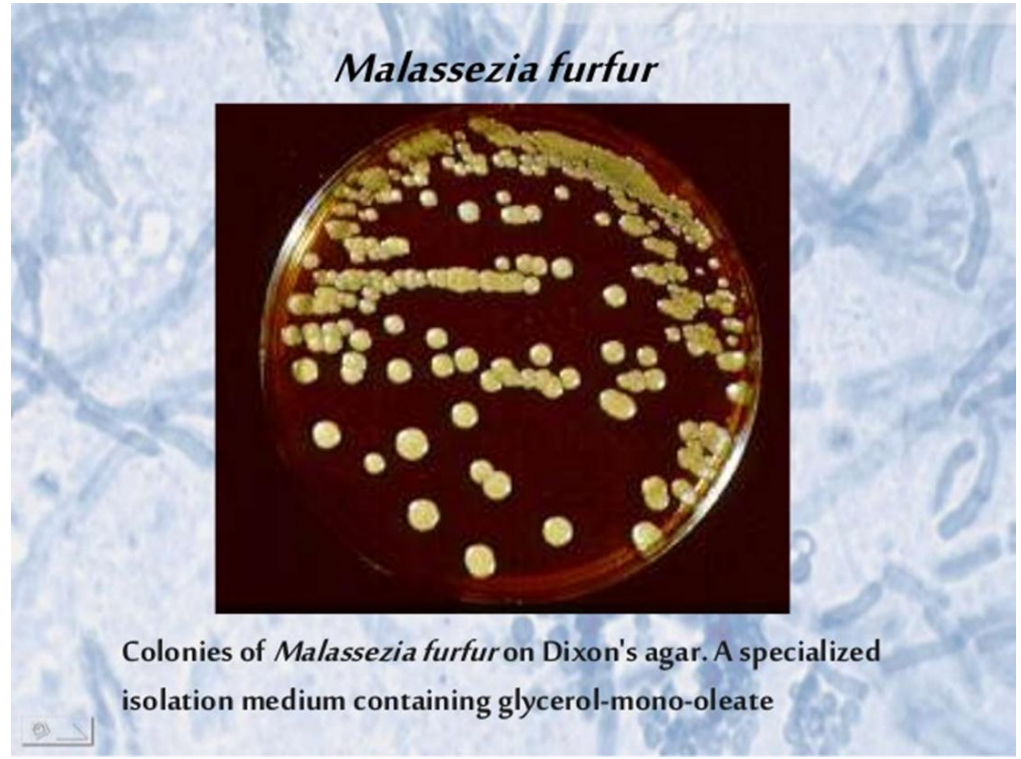


“Spaghetti and meatballs”



Culture

❖ Malassezia furfur can be grown in a laboratory but only when one covers the culture with oil. This leads me to speculate that there is a genetic susceptibility to this disease resulting from qualitative or quantitative differences in the oil content of susceptible persons.

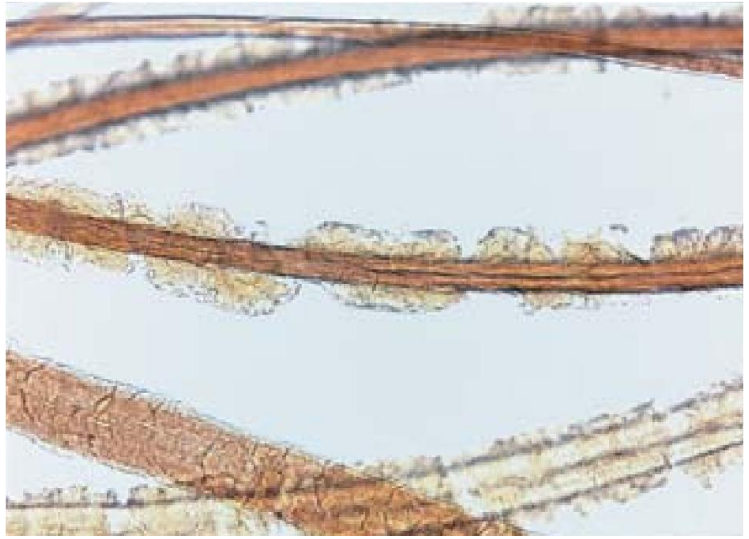


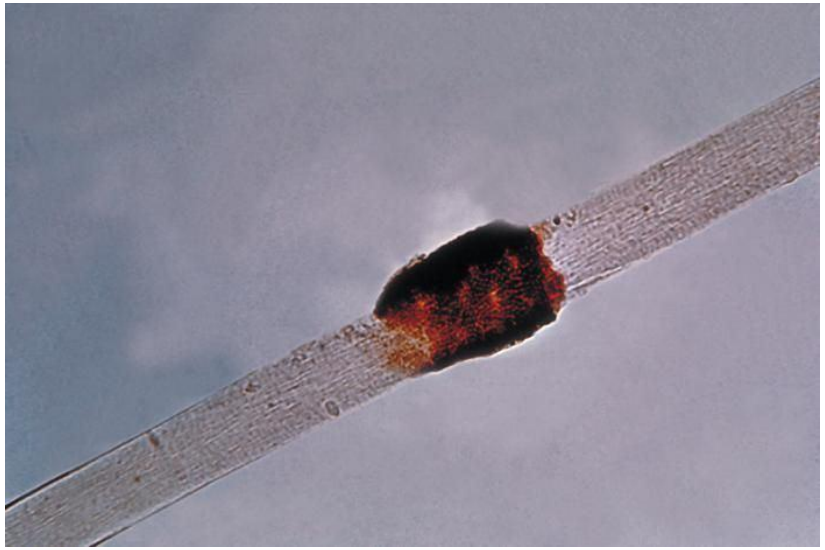
Colonies of *Malassezia furfur* on Dixon's agar. A specialized isolation medium containing glycerol-mono-oleate

	Black piedra	White piedra
Synonyms	Tinea nodosa, trichomycosis nodularis	Trichosporosis nodosa
Clinical features	Firmly adherent, black, gritty hard nodules	Soft, white or light brown, transparent, easily detachable nodules
Site of involvement	Mostly scalp Beard, moustache, pubic area: Less frequent	More commonly: Hairs of beard, moustache and genital areas Scalp: Less common
Causative organism	*Piedraia hortae*	Genus *Trichosporon* Scalp white piedra: *Trichosporon ovoides* Crural white piedra: *Trichosporon inkin*, *Trichosporon asahii*, *Trichosporon mucoides* Possible synergistic role of *Brevibacterium* (proteolytic activity)
Hair shaft breakage	Subcuticular fungal growth can cause rupture of cuticle and hair breakage occurs in longstanding cases	Growth of the fungus both within and outside the hair shaft may cause the hair shaft to weaken and break off
Geographical area	Mostly tropical areas	Mostly tropical areas, but found in temperate climates as well
KOH mount	Nodules composed of closely packed brown, dematiaceous hyphae with arthroconidia 4-8 µm in diameter at the edges of the nodule. The thick parts of the mature nodule show club shaped asci containing 8 elongated ascopores	Nondematiaceous, loosely arranged septate hyphae with arthroconidia (2-4 µm) and budding blastoconidia
Culture characteristics	Slow growth of compact, domed black colonies	Rapid growth of soft, creamy and wrinkly, sometimes mucoid colonies
Microscopic examination of culture isolate	Brown, thick walled, septate hyphae and chlamydoconidia Asci and ascospores in thicker portion of the colony	Hyphae, arthroconidia, budding cells. Physiological tests and genetic analysis required to differentiate the species

KOH – Potassium hydroxide


White piedra
Trichosporon asahii
Black piedra
Piedraia hortae





Cutaneous Mycoses

- **Dermatophytosis** - "ringworm" disease of the nails, hair, and/or stratum corneum of the skin caused by fungi called dermatophytes.
- **Dermatomycosis** - more general name for any skin disease caused by a fungus.

Cutaneous mycoses

- Infection of the skin, hair or nails caused by a group of keratinophilic fungi, called dermatophytes
- **DERMATOPHYTOSIS (=Tinea = Ringworm)**

DERMATOPHYTES

- Digest keratin by their keratinases
- Resistant to cycloheximide
- Classified into three groups depending on their usual habitat

Cutaneous mycoses

- Keratophilic – use keratin as subject to live (parasites)
- Keratinases- invade only keratinized layers

DERMATOPHYTOSIS

Classification

Three Groups/Types:

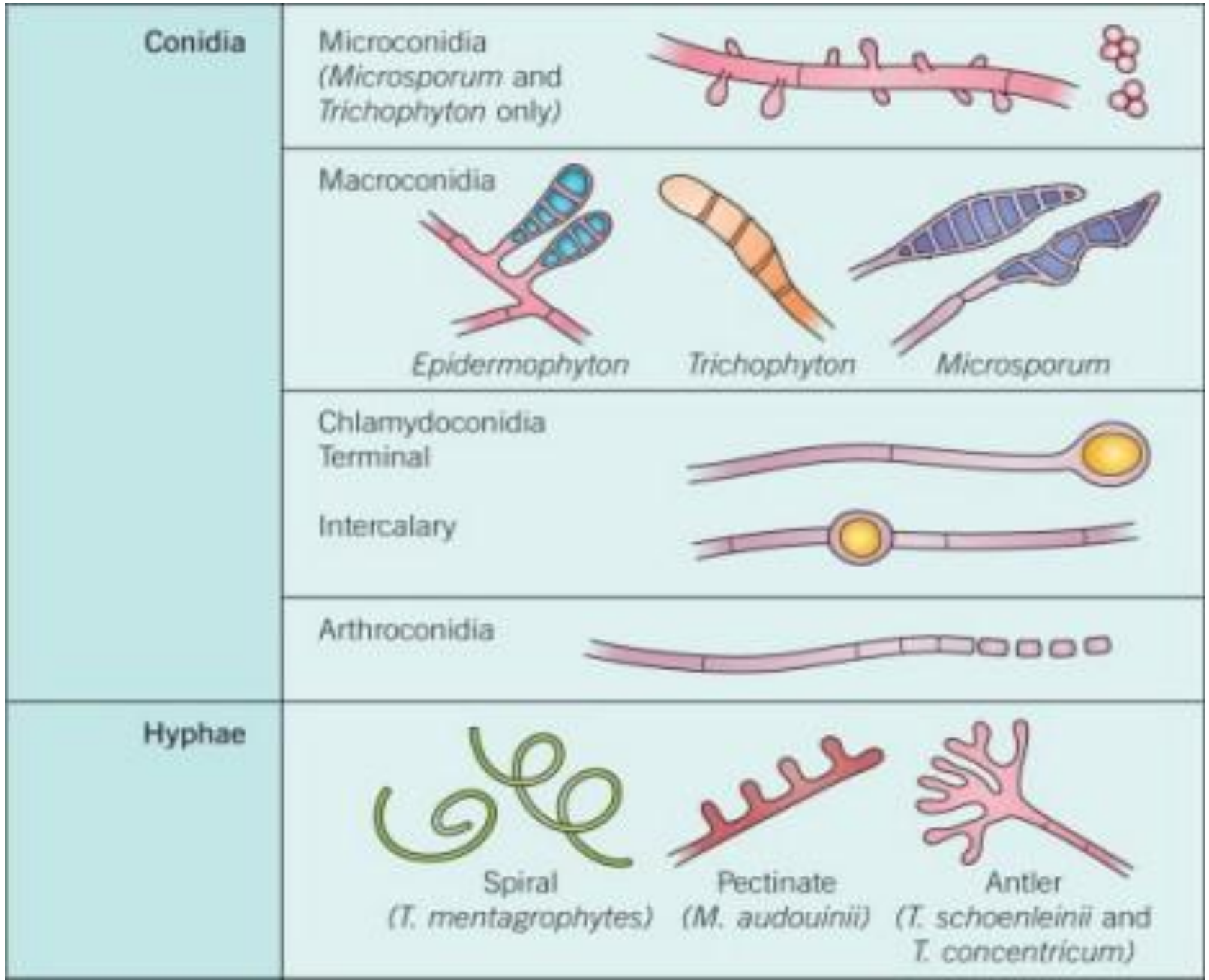
- **Clinical**
- **Etiological**
- **Ecological**

Etiology of Dermatophytes

Etiology (3 Genera)

- Trichophyton
- Microsporum
- Epidermophyton

Features	Trichophytom	Microsporum	Epidermophyton
Site of infection	Skin, hair, nail	Skin, hair	Skin, nail
Macroconidia	Sparse, thin-walled, smooth, Pencil shaped	Numerous, thick-walled, rough, Spindle shaped	Numerous, smooth-walled, Club shaped
Microconidia	Abundant	Rare	Absent
Examples	T.rubrum, T.schoenleinii, T.violaceum, T.concentricum, T.mentagrophytes, T.tonsurans, T.equinum	M.canis, M.fulvum, M.nanum, M.andouinii, M.gypseum, M.racemosum, M.equinum, M.gallinae	E.floccosum


Conidia
Microconidia
(Microsporum and
Trichophyton only)
Macroconidia
Epidermophyton
Trichophyton
Microsporum
Chlamydoconidia
Terminal
Intercalary
Arthroconidia
Hyphae
Spiral
(T. mentagrophytes)
Pectinate
(M. audouinii)
Antler
(T. schoenleinii and
T. concentricum)

Trichophyton mentagrophytes

❖Colony growth is moderately rapid, powdery to granular, white to cream colored on the surface with a yellowish, brown or red-brown reverse.

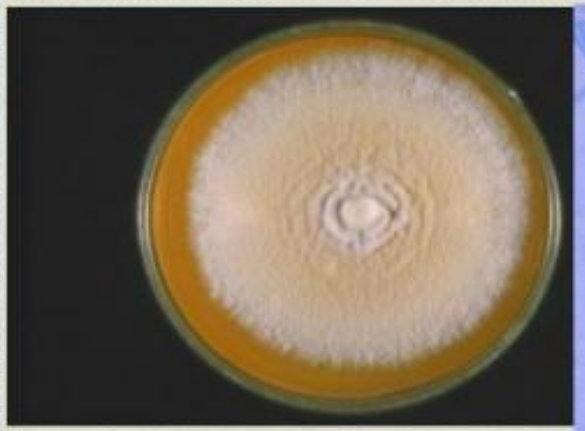
❖ Microconidia are numerous, unicellular, round to pyriform and found in grape like clusters. Spiral hyphae are often present.

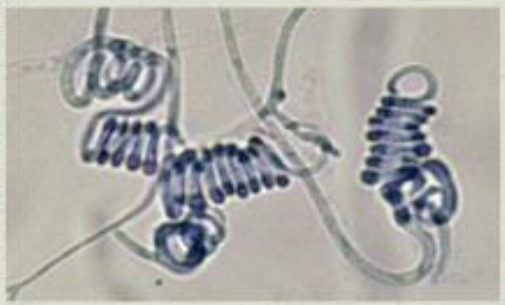
❖Macroconidia are multiseptate, club-shaped and often absent.

❖Lab tests: hair perforation test positive, urease positive, growth at 37°C.

❖Infection is typically found on the feet, hands, or groin, but can also be associated with inflammatory lesions of the scalp, nails, and beard.

Trichophyton mentagrophytes



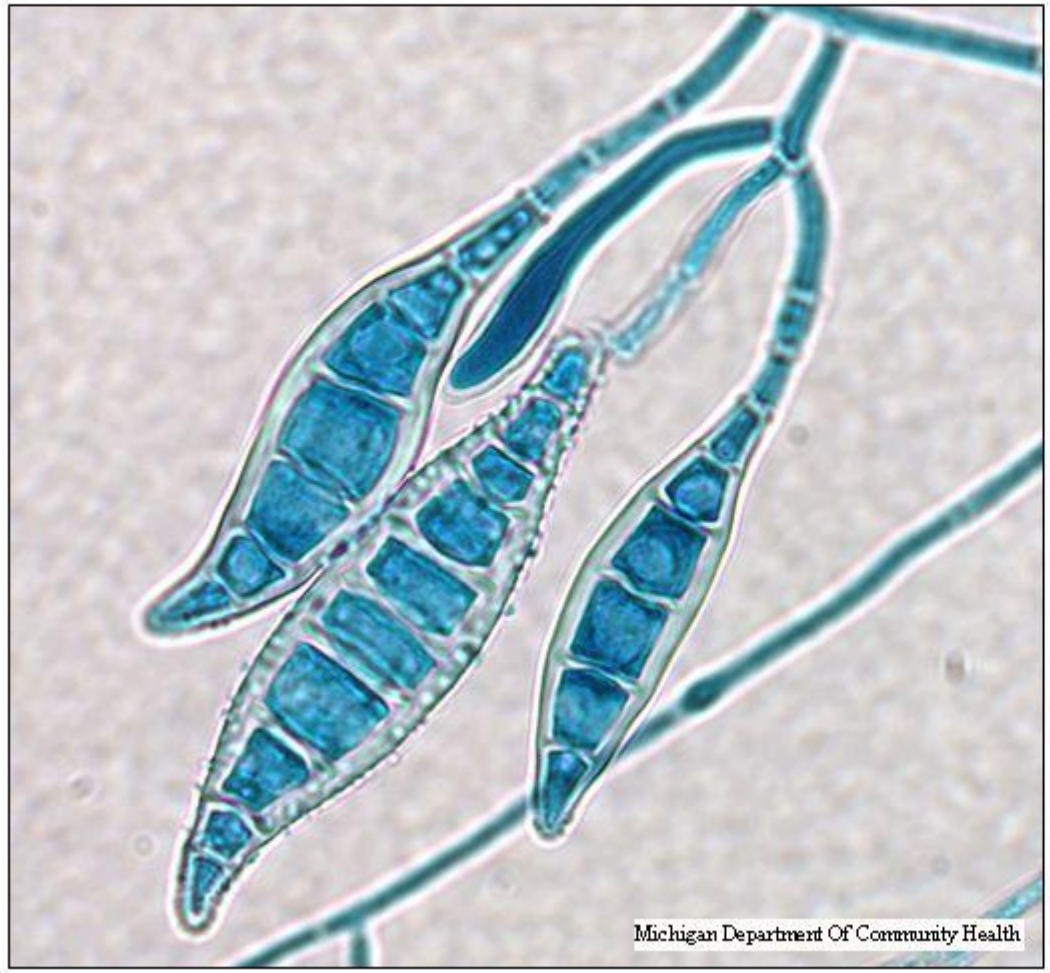


all types of tinea = spiral hyphae "en grappe"

Trichophyton rubrum

- Colony growth is slow to moderate, downy, white on the surface with a red to brown reverse.
- Microconidia are club-shaped to pyriform and are formed along the sides of the hyphae.
- Macroconidia are pencil-shaped to cigar-shaped.
- Lab tests: hair perforation test negative, urease negative, growth at 37°C.
- Infection is typically found on the feet, hands, nails, or groin.

Microsporum canis



❖Colony growth is rapid, downy to wooly, cream to yellow on the surface with a yellow to yellow-orange reverse.

❖Microconidia are club-shaped but typically are absent.

❖Macroconidia are fusoid, verrucose, and thick walled. They have a recurved apex and contain 5-15 cells.

❖Lab tests: hair perforation test positive and urease positive.

❖Infection in humans occurs on the scalp and glabrous skin. It is also a cause of ringworm in cats and dogs.

Microsporum gypseum

❖Colony growth is rapid, downy, becoming powdery to granular, cream, tawny-buff, or pale cinnamon on the surface with a beige to red-brown reverse.

❖Microconidia are moderately abundant and club-shaped.

❖Macroconidia are abundant, ellipsoidal to fusiform, sometimes verrucose, and thin walled. They typically contain 3-6 cells.

❖Lab tests: hair perforation test positive and urease positive.

❖Infection in humans is found on the scalp and glabrous skin; it is more frequently isolated from the soil and from the fur of small rodents.

Microsporum gypseum

Epidermophyton floccosum

❖Colony growth is slow, powdery, with a yellow to khaki surface color and chamois to brown reverse.

❖Macroconidia are club shaped, with thin smooth walls and can be solitary or grouped in clusters. Chlamydospores are often produced in large numbers.

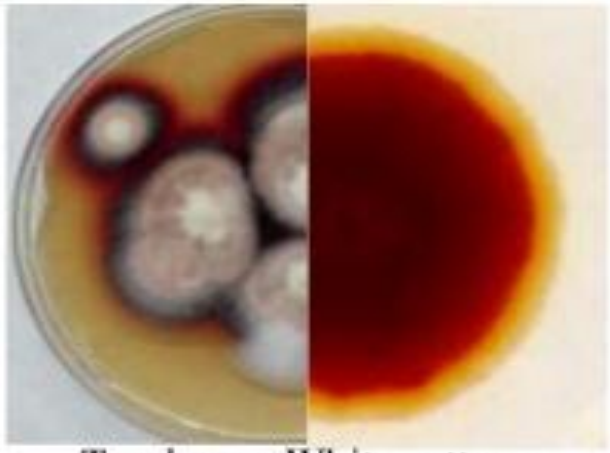
❖Microconidia are absent.

❖Lab tests: hair perforation test negative, urease positive, growth at 37°C.

❖Infections are commonly cutaneous, especially of the groin or feet.

Epidermophyton floccosum

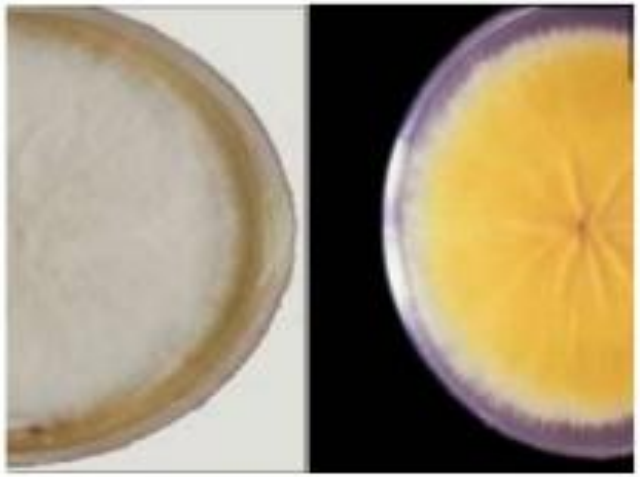
- Only one pathogenic species in this genus.
- **Tinea unguium and tinea cruris** are often caused by this fungus.



T. rubrum : White cottony surface and a deep red nondiffusible pigment from reverse side

T. tonsurans : flat, powdery, velvety colony.

T. metagrophytes : cottony to granular colony



Microsporum : white cottony sarface with deep yellow from revese.

Epidermophyton : flat, velvety with a tan to olive green tinge.

Dermatophyte test media

- **Diseases:**
  - **Hairy areas:**
    - Tinea capitis — Tinea barbae
  - **Skin:**
    - Tinea corporis — Tinea cruris
    - Tinea manum — Tinea pedis
    - Tinea fascie — Tinea imbricata
  - **Nail:**
    - Tinea ungium

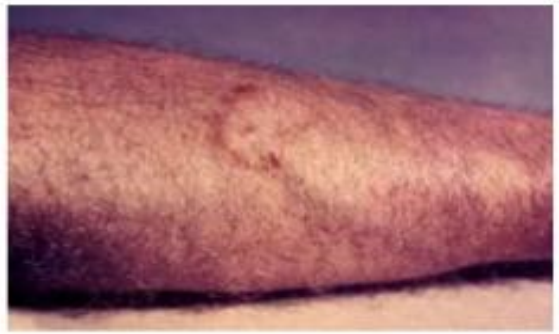
Clinical Dermatophytosis

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 barbae:

ringworm of the bearded areas of the face and neck.

- **Tinea unguium (onychomycosis) :**

nails. Clipped and used for culture

- **Tinea capitis :**

head. Frequently found in children

Ecology of Dermatophytes

To determine the source of infection

- Anthropophilic
- Zoophilic
- Geophilic

Anthropophilic

• Associated with humans only. Person -to-person transmission through contaminated objects (comb, hat, etc.)

• e.g., *M. audounii*, *T. tonsurans*

Zoophilic

• Associated with animals. Direct transmission to humans by close contact with animals.

• e.g., *M. canis*, *T. verrucosum*

Geophilic

• Usually found in soil (soil saprophytes). Transmitted to humans by direct exposure.

•e.g., *M. gypseum*, *T. ajelloi*.

DERMATOPHYTOSIS
Diagnosis

I. Clinical

Appearance

Wood's lamp (UV, 365 nm)

II. Lab

A. Direct microscopic examination

(10-25% KOH)

DERMATOPHYTOSIS
Diagnosis

B. Culture

- Mycobiotic agar
- Sabouraud dextrose agar
- Selective media – containing cycloheximide and chlorampenicol→incubate at 25 C.
- Identification based on the conidia

SUBCUTANEOUS MYCOSES

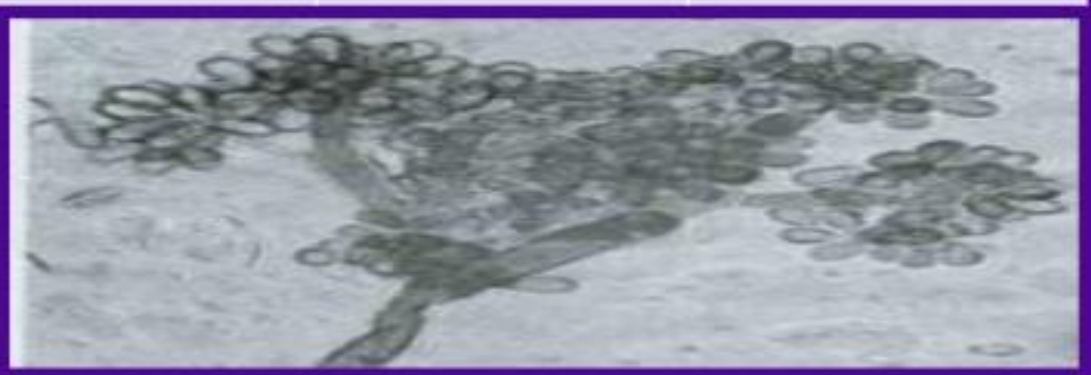
- Main
  - Lymphocutaneous sporotrichosis
  - Chromoblastomycosis
  - Eumycotic mycetoma
  - Subcutaneous zygomycosis
  - Subcutaneous phaeohyphomycosis

- Additional
  - Lobomycosis
  - Rhinosporidiosis

SUBCUTANEOUS MYCOSES	CAUSITIVE ORGANISMS	CLINICAL SYNDROME	DIAGNOSIS	TREATMENT
1. LYMPHOCUTANEOUS SPOROTRICHOSIS	*Sporothrix schenckii – thermophilic dimorphic Mold – narrow, hyaline, septate – condiophores Yeast – spherical, oval (or) elongated*	➢Skin by truma ➢Small nodule – ulcerate ➢Then discharge pus	➢Culture of pus / tissue ➢Immunologically – exoantigen test	➢ Oral potassium iodide ➢Itraconazole, terbinafine /potassium iodide ➢Fluconazole ➢Local application of heat

SUBCUTANEOUS MYCOSES	CAUSITIVE ORGANISMS	CLINICAL SYNDROME	DIAGNOSIS	TREATMENT
2. CHROMOBLASTOMYCOSIS (Chromomycosis)	*Pigmented septated (dematiaceous) fungi* *Fonsecaea, Cladosporium, Exophiala, Cladophialophora Rhinocladiella Phialophora* *Form muriform cells (Sclerotic bodies, Medlar bodies)*	➢Legs & arms/ shoulds, neck, truck, face, ears. ➢Chronic, pruritic, progressive, resistant to treat. ➢Verrucous lesions to flat plagues ➢Cauliflower like growth	➢Histopathologic findings of chestnut brown, musiform cells.	➢ antifungal theraphy Itraconazole & terbinafine ➢Posaconazole with flucytosine

SUBCUTANEOUS MYCOSES	CAUSITIVE ORGANISMS	CLINICAL SYNDROME	DIAGNOSIS	TREATMENT
3. EUMYCOTIC MYCETOMA	*Phaeoacremonium* *Curvularia, Fusarium Madurella, Exophiala, Pyrenochaeta, Leptosphaeria Scedosporium spp.* *Septate Chlamydoconidia*	➢ Foot & hand/back shoulders & chest wall also. ➢ Small, painless, subcutaneous nodule/plague - increase slowly & enlarge - disfigured	➢ Grains can be mounted ➢ Microscopic	➢ Amphotericin B, Kitoconazole, itraconazole ➢ Antifungal Theraphy



MYCETOMA- causative agents

FUNGI	ACTINOMYCETES
Madurella mycetomatis	*Actinomadura madurae*
Madurella grisea	*Actinomadura pelletieri*
Leptosphaeria senegalensis	*Nocardia brasiliensis*
Neotestudina rosatii	*Nocardia asteroides*
Fusarium moniliforme	*Streptomyces somaliensis*
Fusarium solani	

Madura foot

SYSTEMIC MYCOSES

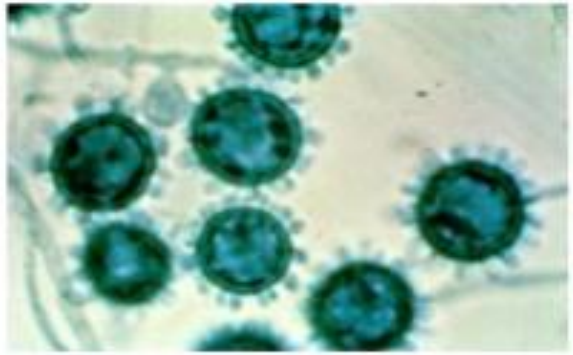
TRUE PATHOGENIC FUNGI

Genus	Species	Clinical conditions
Histoplasma	Histoplasma capsulatum	Histoplasmosis
Coccidiodies	Coccidioides immitis	coccidiodimycosis
Blastomyces	Blastomyces dermatitidis	blastomycoses
Paracoccidioides	Paracoccidioides braziliensis	paracoccidiomycosis

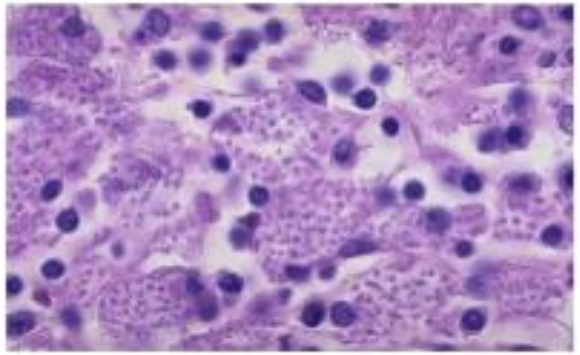
What does Dimorphic mean?

- Two forms exist for one fungus species depending on temperature and conditions of environment
  - Mycelial form - Hyphae and conidia
    - free living form found in nature and at laboratory temperature <=30˚C
  - Yeast or yeast like form
    - parasitic phase found in human tissue or in the lab >= 35˚

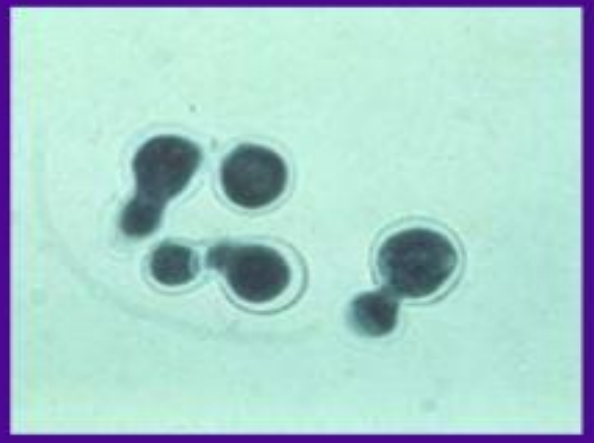
Histoplasma capsulatum – mold from 30˚C culture

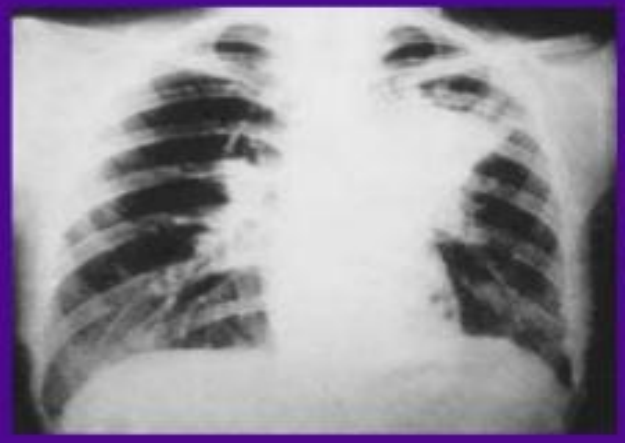


Histoplasma capsulatum – yeast from tissue and 35˚C culture



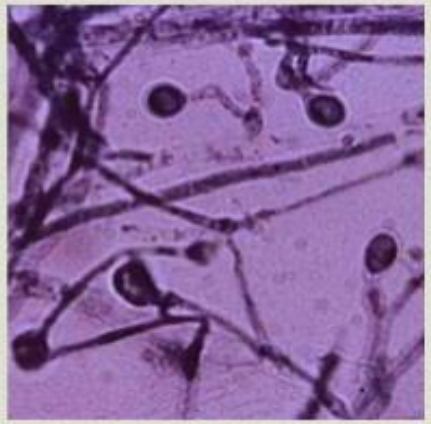
SYSTEMIC MYCOSES	CAUSITIVE ORGANISMS	CLINICAL SYNDROME	DIAGNOSIS	TREATMENT
1. BLASTOMYCES DERMATITIDIS	BLASTOMYCOSIS Molds in culture - Branced septate Yeast like in tissue. – single budding yeast like *Older culture may produce chlamydospores*	➢Present as pulmonary disease (or) extra pulmonary disseminated disease ➢Asymptomatic /present as a mild flu like illness.	➢Suptum,pus , exudates, Urine & biopsis from lessions. ➢Microscopic examination ➢Culture ➢Seriologic test	➢Itraconazole ➢Ketoconazole ➢Serious cases amphotericin B.

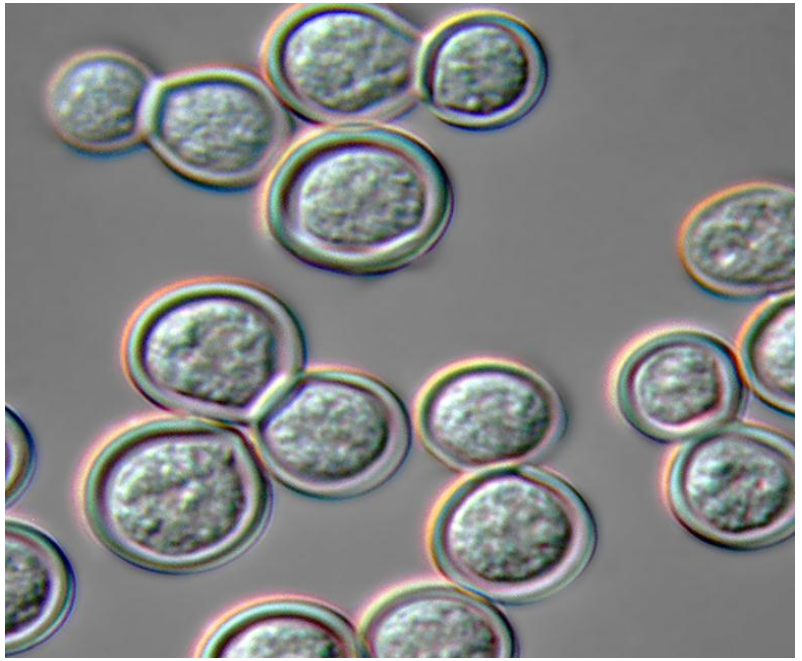




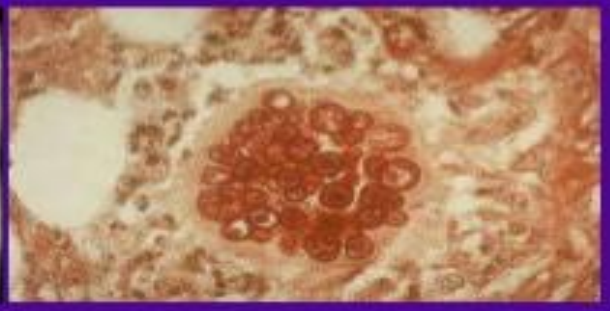
Blastomyces dermatitidis

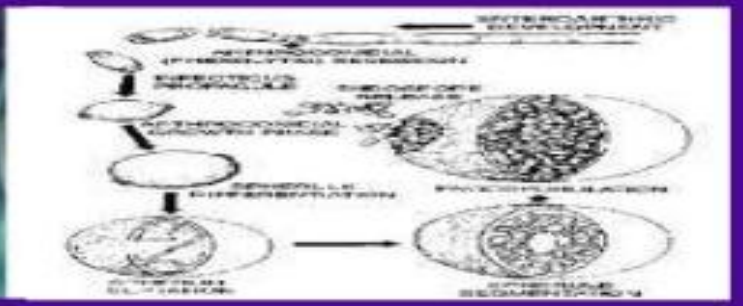
- **MYCELIAL FORM**
  - The mycelial phase at 25° C showed typical pyriform microconidia , which are about 2-4 microns in diameter.
- **YEAST FORM**
  - At 37° C and at body temperature, this organism is a yeast 8-15 microns in diameter.
  - Buds are produced singly and are attached to parent cell by broad base.





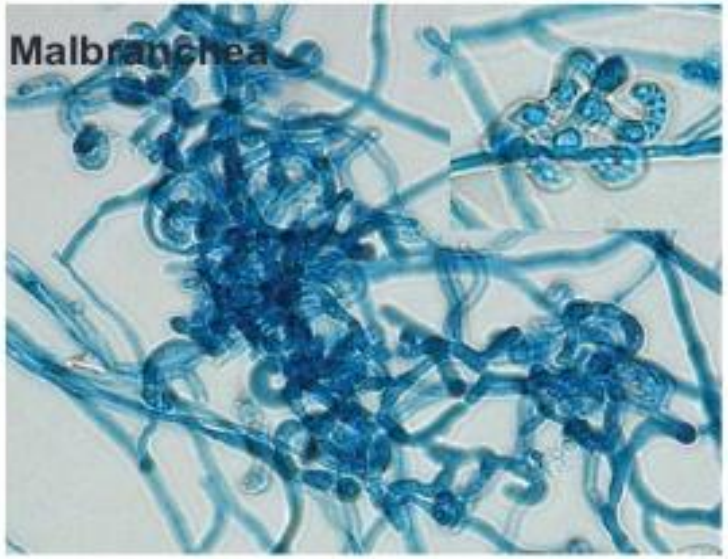
SYSTEMIC MYCOSES	CAUSITIVE ORGANISMS	CLINICAL SYNDROME	DIAGNOSIS	TREATMENT
2.COCCIDIOIDOMYCOSIS	*Coccidioides immitis* & *C.posadasii* Molds hyphae – arthroconidia – endosporulating spherule in tissue.	➢Asymptomatic pulmonary disease ➢Vally fever/SanJoaquin vally fever/desert rheumatism	➢Suptum, exudates from cutaneous lession, spinal fluid, blood, Urine & Tissue biopsis. ➢Microscopic examination ➢Culture ➢Seriologic test	➢Itraconazole ➢Serious cases amphotericin B. ➢Azoles, fluconazole



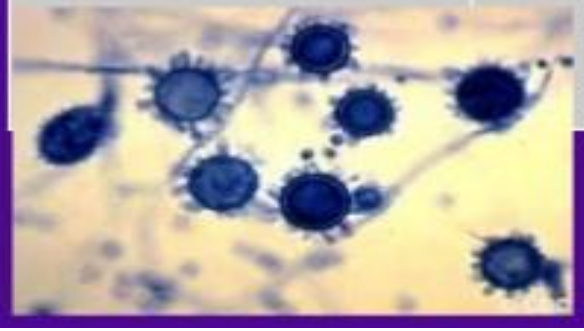


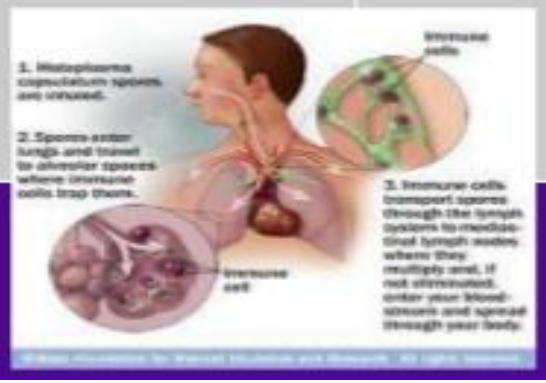
Coccidioides

- Malbranchea species can look like C. immitis under the microscope
- Because of look-a-like fungi one needs to confirm identification of Coccidioides immitis with DNA probe or similar method to be sure!

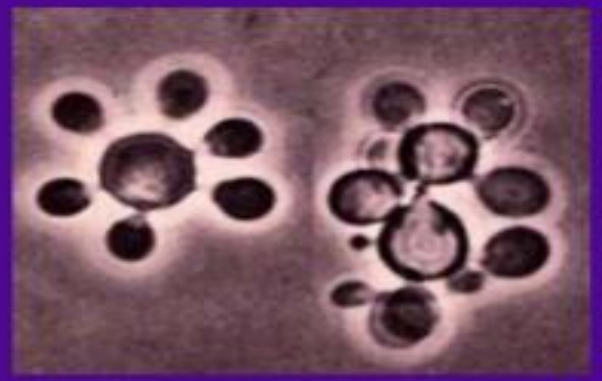


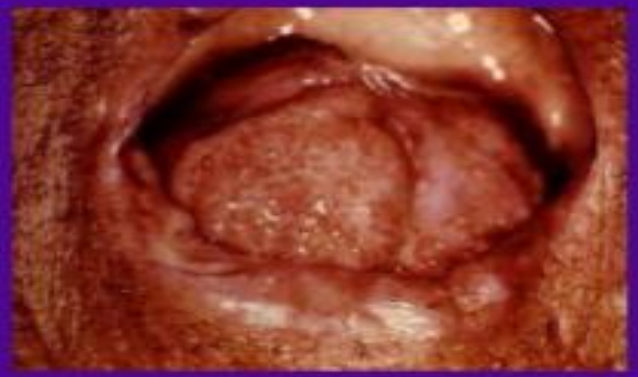
SYSTEMIC MYCOSES	CAUSITIVE ORGANISMS	CLINICAL SYNDROME	DIAGNOSIS	TREATMENT
3.HISTOPLASMOSIS	Histocapsulatum var Capsulatum, H. capsulatum var duboisii Molds in culture - hyaline septate - conidiophore Yeast like in tissue. – intracelluar budding yeast like	➢Conidia develops into yeast cells – engulfed by alvelor macrophages ➢Initial inflammatory reaction because granulomatous ➢Mediastinal fibrosis	➢Suptum, scrapings from superficial lesions, bone marrow, aspirates & buffy coat blood cells, blood films & biopsis from lessions. ➢Microscopic examination ➢Culture ➢Seriologic test	➢Itraconazole ➢In disseminated disease, treatment with amphotericin B.

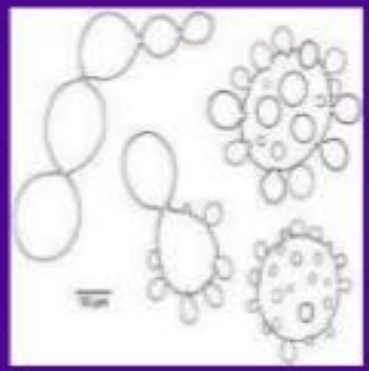




SYSTEMIC MYCOSES	CAUSITIVE ORGANISMS	CLINICAL SYNDROME	DIAGNOSIS	TREATMENT
4.PARACOCCIDIOIDOMYCOSIS	Paracoccidioides brasiliensis Molds in culture - Branced septate Yeast like in tissue. – multiply budding yeast like *Older culture may produce chlamydospores and conidia*	➢Present as pulmonary disease (or) extra pulmonary disseminated disease.	➢Suptum, exudates & biopsis from lessions. ➢Microscopic examination ➢Culture ➢Seriologic test	➢Itraconazole ➢Ketoconazole & Trimethoprim-sulfamethoxazole ➢Serious cases amphotericin B.







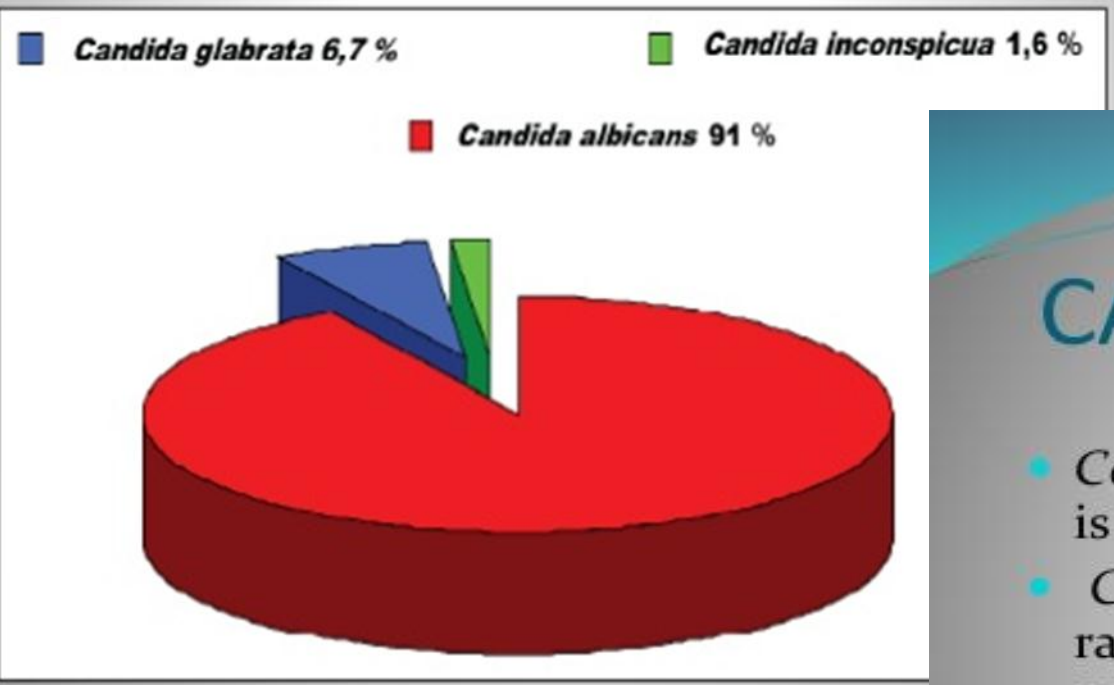
Opportunistic mycoses

Causative agents

- *Candida* species
- *Cryptococcus neoformans*
- *Aspergillus species*
- *Zygomycosis (Rhizopus, mucor, absidia)*
- *Penicillium species*
- *Fusarium species*
- *Alternaria species*

***ANY fungus found in nature may give rise to opportunistic mycoses ***

CANDIDIASIS



CANDIDIASIS

- *Candida albicans* is an opportunistic fungal pathogen that is responsible for candidiasis in human hosts.
- *C. albicans* grow in several different morphological forms, ranging from unicellular budding yeast to true hyphae with parallel-side wall .
- Candida albicans is a unicellular, oval-shaped diploid fungus (a form of yeast)
- Typically, *C. albicans* live as harmless commensals in the gastrointestinal and genitourinary tract and are found in over 70% of the population. Overgrowth of these organisms, however, will lead to disease

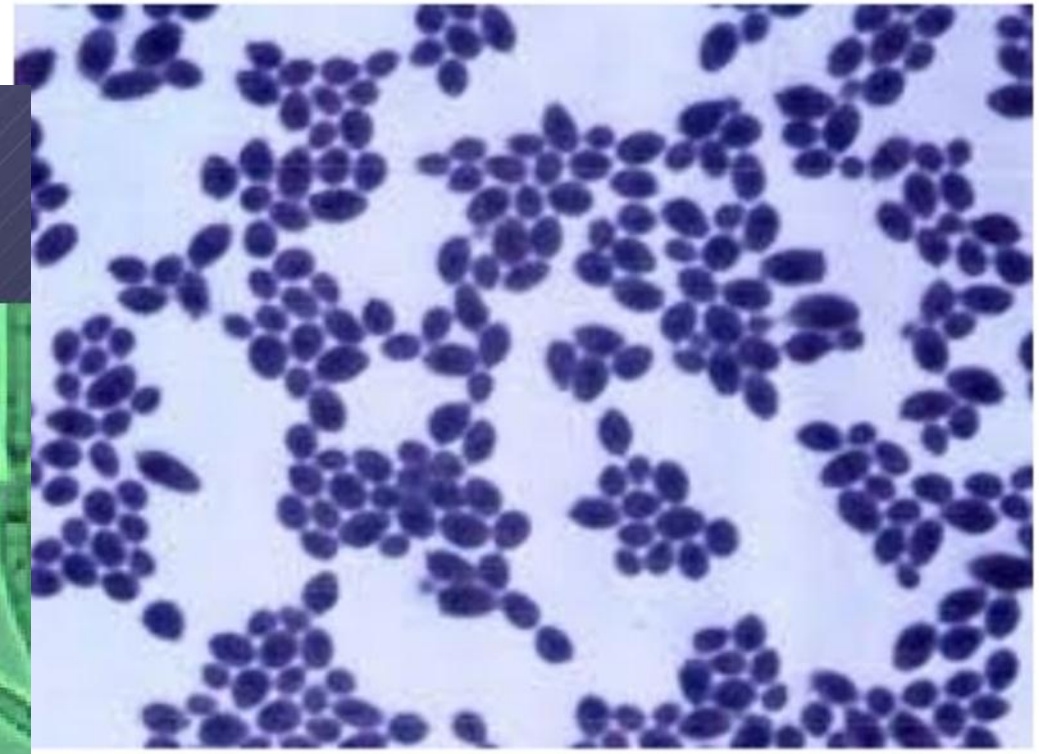
Morphology and Culturing

- Ovoid shape or spherical budding cells and produces pseudo mycelium
- Routine cultures are done on Sabouraud's Glucose agar,
- Grow predominantly in yeast phase
- A mixture of yeast cells and pseudo mycelium and true mycelium are seen in Vivo and Nutritionally poor media.

Macroscopic and Microscopic appearance of Candida spp

STRUCTURE OF C.ALBICANS

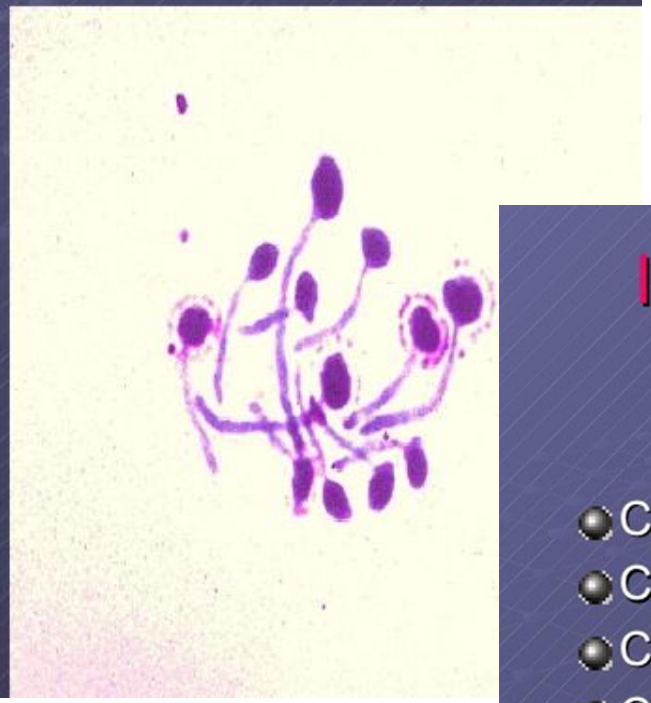
GRAM STAIN; CANDIDA YEAST



Pseudohypal structures in Candida

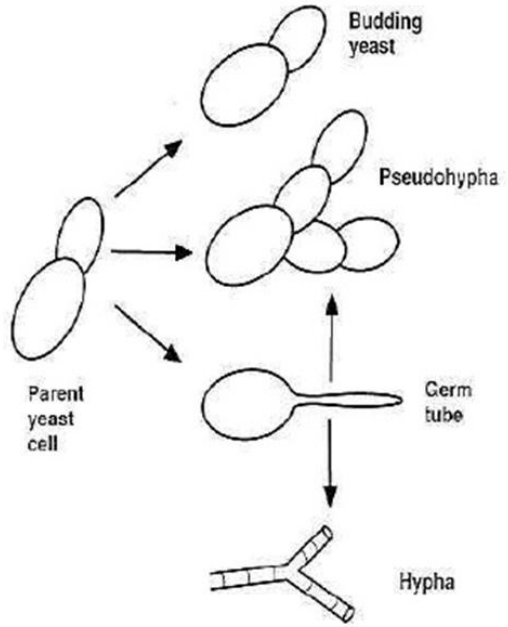
Easier Identification of species as C.albicans

- ***Germ tube test*** identifies C.albicans from other Candida species.
- Majority of Diagnostic laboratories depend on this test.



Important species of Candida in Human infections

- C.albicans
- C.tropicalis
- C.glabrata
- C.Krusei



Procedure of Germ Tube Test

Put 0.5 ml of sheep or human serum into a small tube.

Note: Fetal bovine serum can also be used instead of human serum.

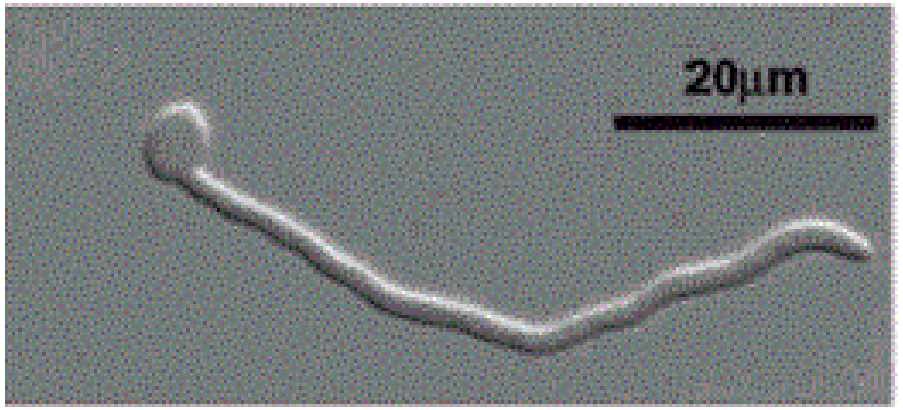
Using a Pasteur pipette, touch a colony of yeast and gently emulsify it in the serum.

Note: Too large of an inoculum will inhibit germ tube formation.

Incubated the tube at 37°C for 2 to 4 hours.

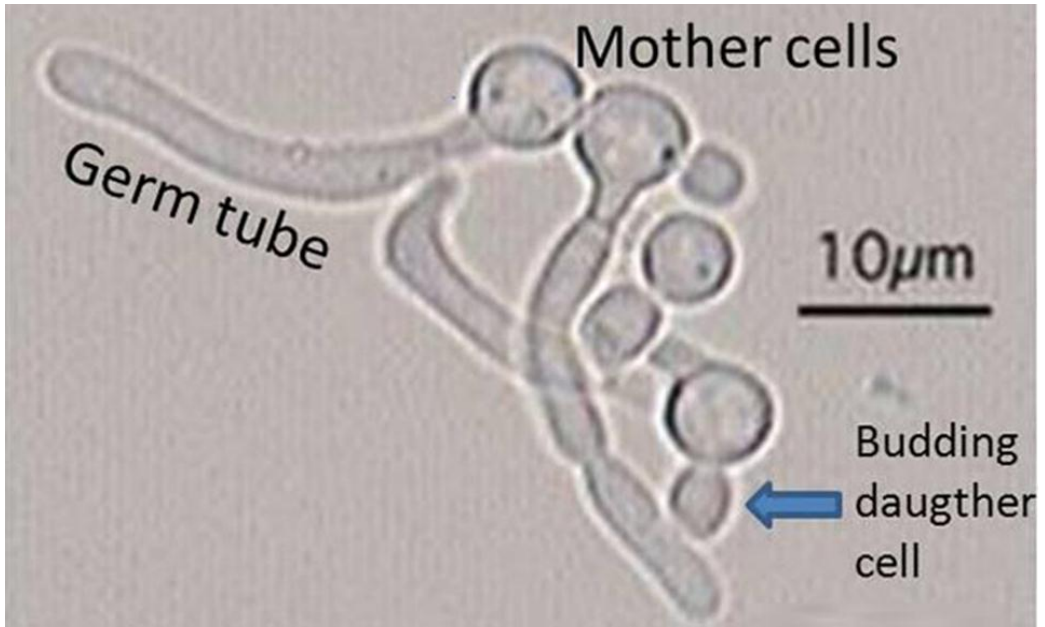
Transfer a drop of the serum to a slide for examination.

Coverslip and examine microscopically under low and high power objectives.



Germ Tube (no constriction)

Pseudohyphae (constriction)



- Candidiasis is a fungal infection that can affect areas such as the:
- Skin
- Genitals
- Throat
- Mouth
- Blood

EPIDEMIOLOGY

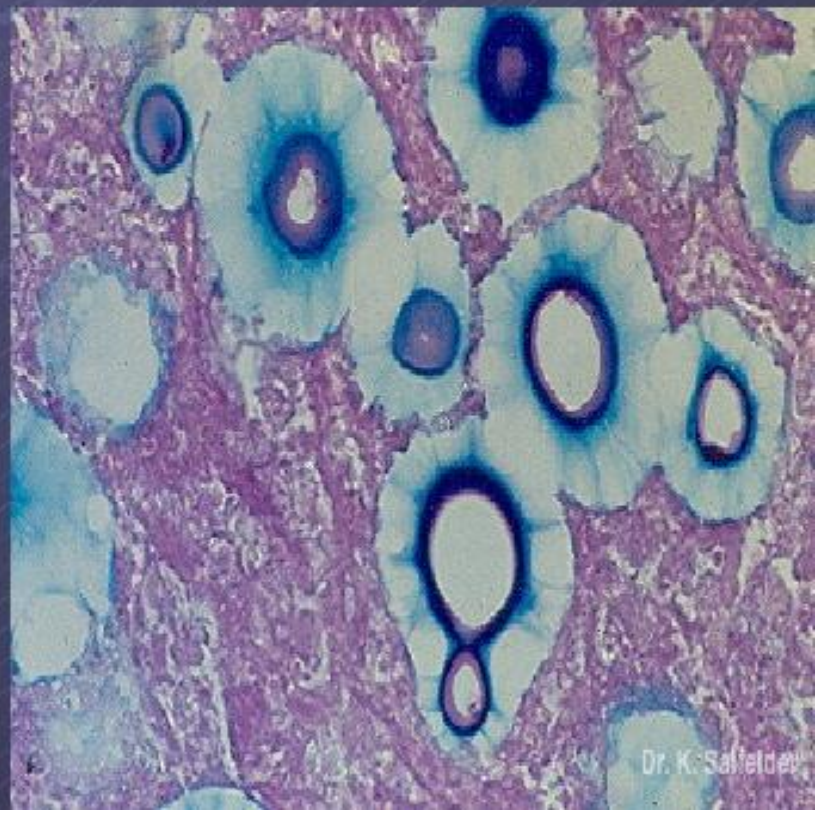
- Over 75% of women suffer from a *C. albicans* infection, usually vulvovaginal candidiasis, in their lifetimes, and 40-50% of them will have additional occurrences(s).
- Interestingly, *C. albicans* are the 4th leading cause for nosocomial infections in patients' bloodstreams.
- This could result in an extremely life-threatening, systemic infection in hospital patients with a mortality rate of 30%

OPPORTUNISTIC PATHOGENS				
Candida species Saprobic and parasitic phases are the same: budding yeast, hyphae, pseudohyphae	Saprobic habitat: ▪ Gastrointestinal mucosa, vaginal mucosa, skin, nails Mode of infection: ▪ Gastrointestinal translocation ▪ Intravascular catheters	Mucosal overgrowth with subsequent invasion; usually impaired mucosal barrier; hematogenous dissemination; transfer from hands of health care worker to catheter hub; catheter colonization and hematogenous dissemination	▪ Growth at 37° C ▪ Bud-hyphae transition ▪ Adherence ▪ Cell-surface hydrophobicity ▪ Cell wall mannans ▪ Proteases and phospholipases ▪ Phenotypic switching	▪ Simple mucosal colonization ▪ Mucocutaneous candidiasis ▪ Oral/vaginal thrush ▪ Hematogenous dissemination ▪ Hepatosplenic candidiasis ▪ Endophthalmitis
Cryptococcus neoformans Saprobic and parasitic phases are the same: encapsulated budding yeast	Saprobic habitat: ▪ Soil enriched with bird (pigeon) guano Mode of infection: ▪ Inhalation of aerosolized yeast ▪ Percutaneous inoculation	Inhaled yeast cells ingested by macrophages; survive intracellularly; capsule inhibits phagocytosis; capsule and melanin protect from oxidative injury; hematogenous and lymphatic dissemination to brain	▪ Growth at 37° C ▪ Polysaccharide capsule ▪ Melanin ▪ Alpha-mating type	▪ Primary cryptococcal pneumonia ▪ Meningitis ▪ Hematogenous dissemination ▪ Genitourinary (prostatic) cryptococcosis ▪ Primary cutaneous cryptococcosis
Aspergillus species Saprobic phase: ▪ Septate mycelium, conidial heads, and conidia Parasitic phase: ▪ Septate mycelium; conidia, and conidial heads usually only seen in cavitary lesions	Saprobic habitat: ▪ Soil, plants, water, pepper, air Mode of infection: ▪ Inhalation of conidia ▪ Transfer to wounds via contaminated tape/bandages	Inhaled conidia bind to fibrinogen and laminin in alveolus; conidia germinate, and hyphal forms secrete proteases and invade epithelium; vascular invasion results in thrombosis and infarction of tissue; hematogenous dissemination	▪ Growth at 37° C ▪ Binding to fibrinogen and laminin ▪ Secretion of elastase and proteases ▪ Catalase ▪ Gliotoxin (?) and other mycotoxins	▪ Allergic bronchopulmonary aspergillosis ▪ Sinusitis ▪ Aspergilloma ▪ Invasive aspergillosis ▪ Lung ▪ Brain ▪ Skin ▪ Gastrointestinal ▪ Heart

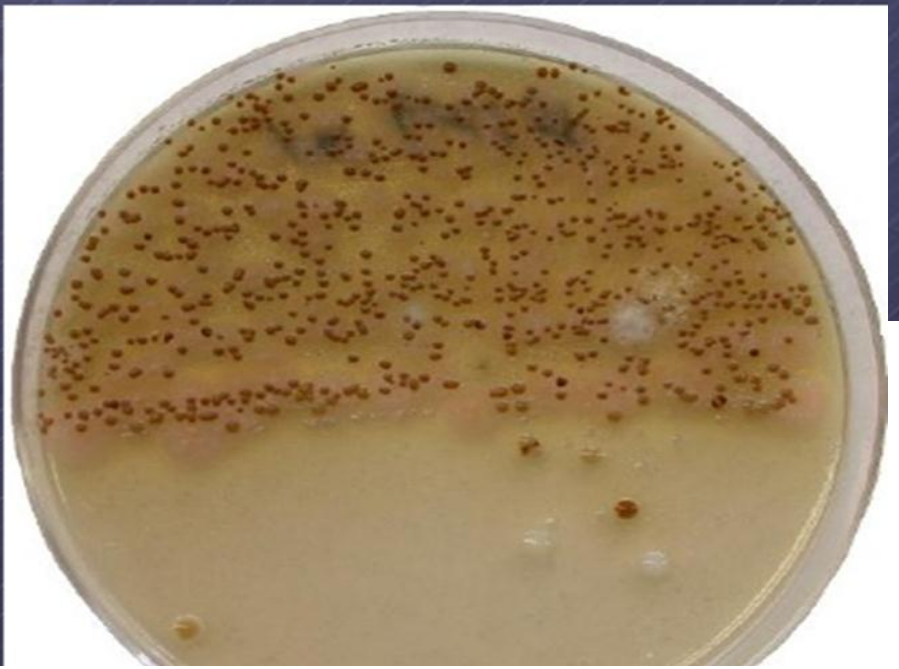
From Cole, G.T., 2003. Fungal pathogenesis. In: Anaissie, E.J., McGinnis, M.R., Pfaller, M.A., (Eds.), Clinical Mycology. Churchill Livingstone, New York.

Cryptococcus neoformans

- A Capsulated yeast – A true yeast..
- A sporadic disease in the past.
- Most common infection in AIDS patients.



- CSF -Culturing on Sabouraud's agar, and incubated at 37° c for upto to 3 weeks
- Cultures appear as Creamy, white, yellow
- Brown colored
- Simple urease test helps in confirming the isolate.

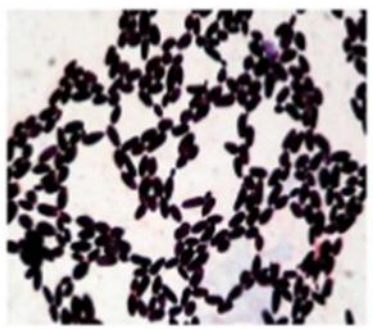


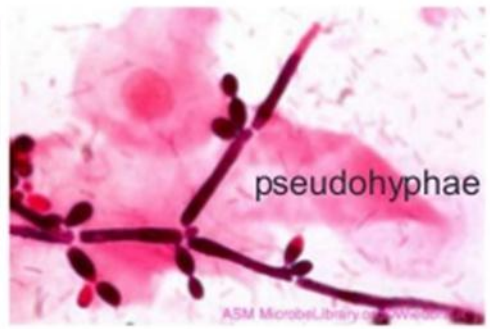
Direct Exams used to identify fungi directly from patient specimens

- **Gram stain** – all specimen types can be Gram stained. Can only reliably detect yeast by Gram stain.
- **KOH preparation** – Skin, Hair or Nails examined for both yeast and/or hyphae
- **Calcofluor white stain** – all specimen types can be stained and examined for yeast and/or hyphae
- **India ink** – Primarily used for CSF for the detection of Cryptococcus neoformans and C. gattii

Gram Stain

Yeast cells stain blue [Gram positive]. Examine for budding cells to confirm that it is a yeast cell and not an artifact. Examination on oil immersion lens.
You can also detect pseudohyphae on Gram stain.
Mold can be difficult to identify on a Gram stain.

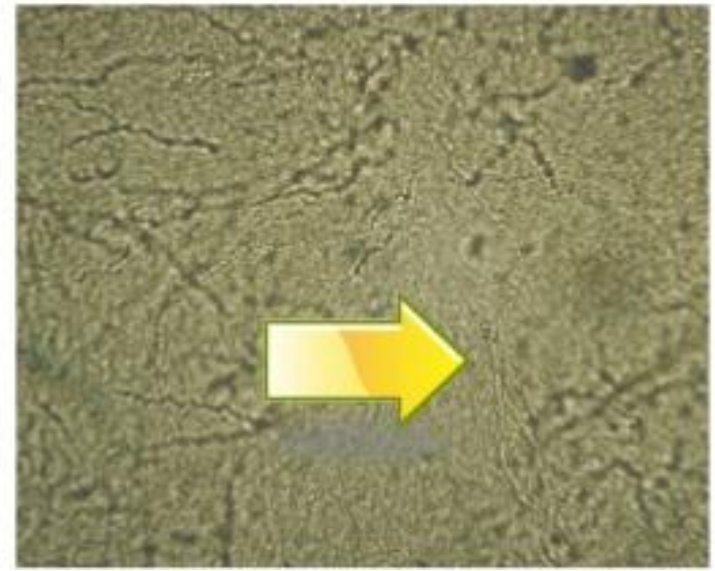




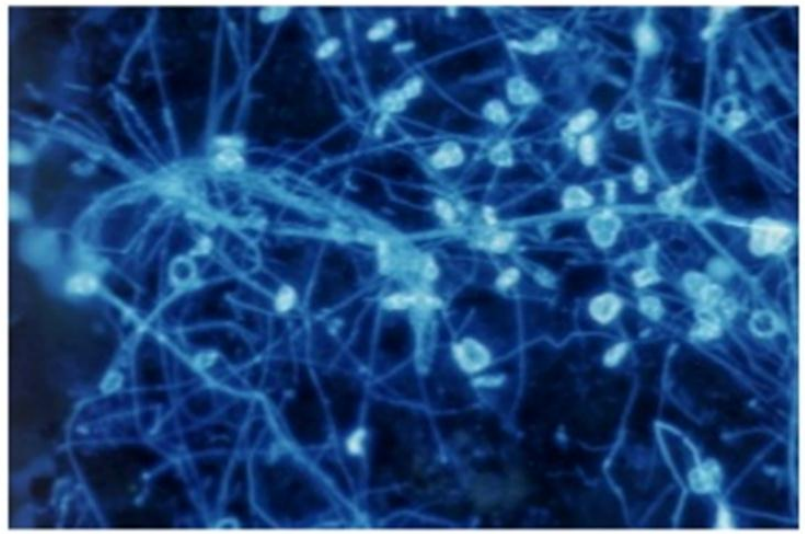
KOH – potassium hydroxide prep

Used to detect yeast and/or hyphae in skin, hair and nail specimens using 40X light microscope.
KOH dissolves keratin found in cell material

and frees hyphae from the cell
KOH exams can be difficult to interpret!



Calcofluor white stain

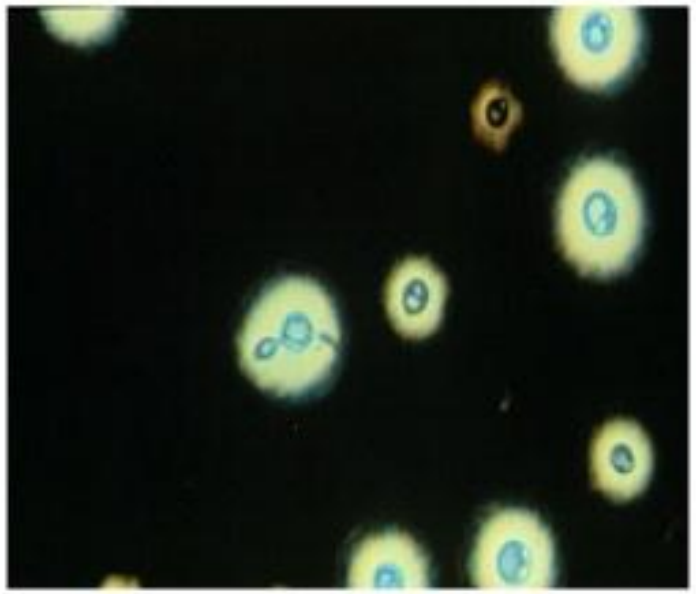


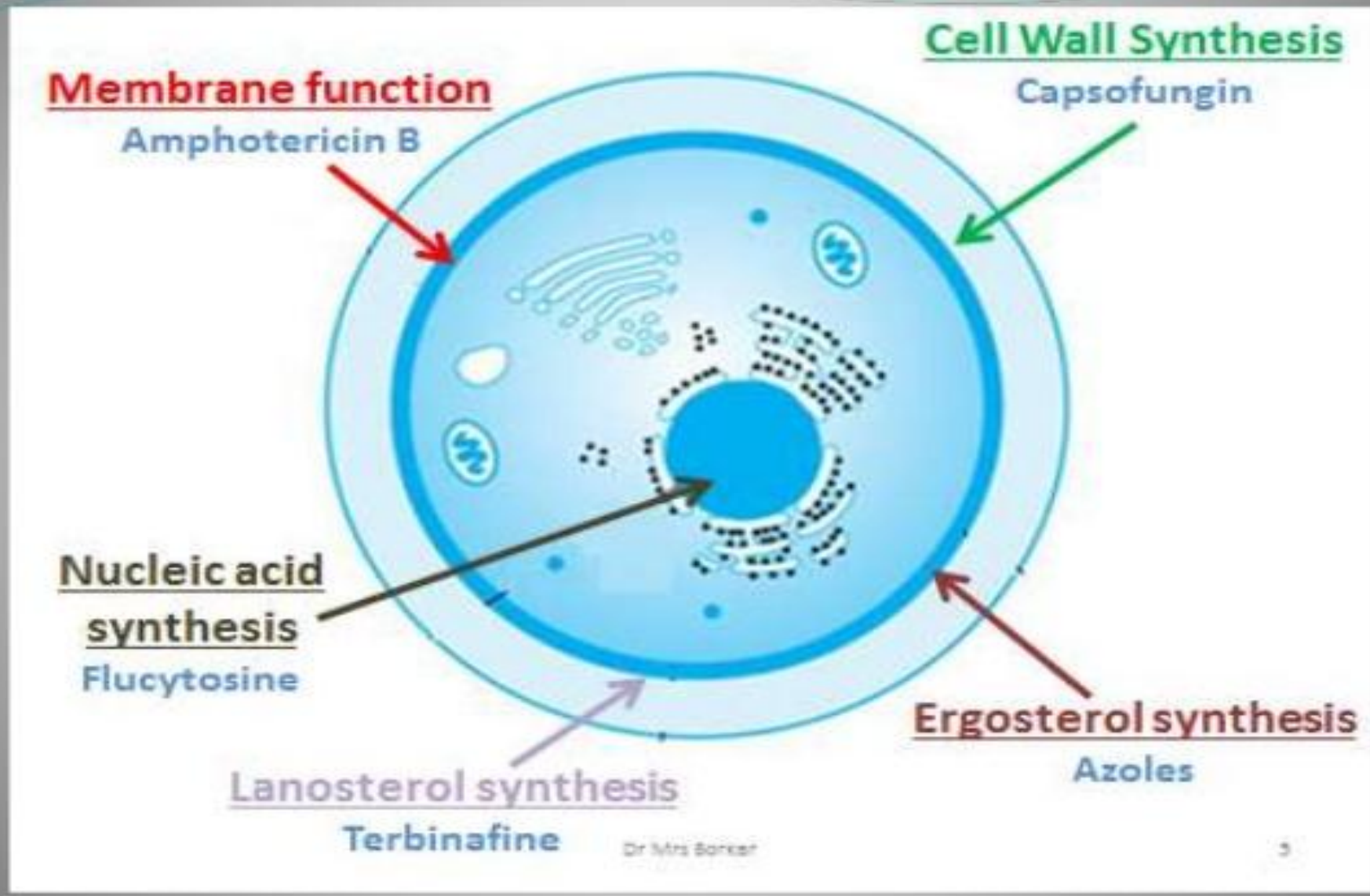
Yeast, pseudohyphae, and mycelial fungi bind with the Calcofluor white stain.
Prep is interpreted using a fluorescence microscope.
Sensitivity and specificity is improved over the KOH preparation.

India Ink

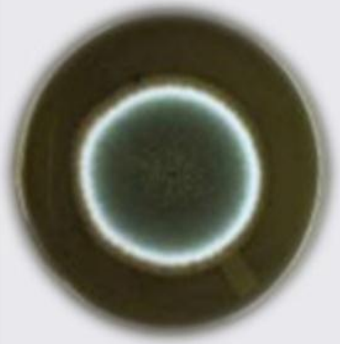
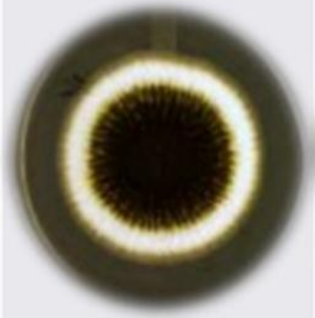
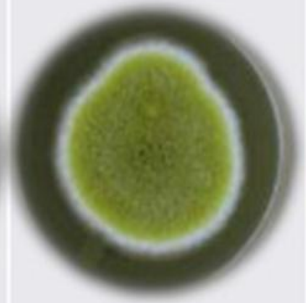
One drop of black ink is placed into one drop of CSF and examined using a 40X lens on light microscope

It is a “negative” stain, staining the background not the yeast cell
The clearing is the polysaccharide capsule of Cryptococcus neoformans or C. gattii. Specificity is improved if you look for budding yeast cells.



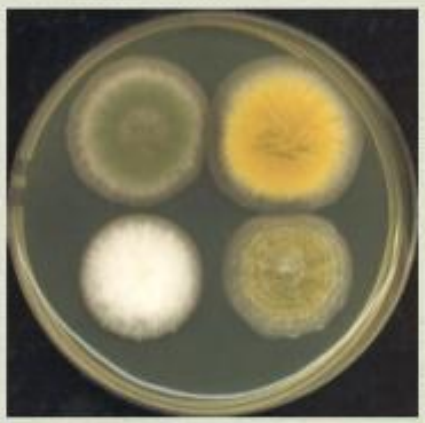

Cell Wall Synthesis
Capsofungin
Membrane function
Amphotericin B
Nucleic acid synthesis
Flucytosine
Ergosterol synthesis
Azoles
Lanosterol synthesis
Terbinafine

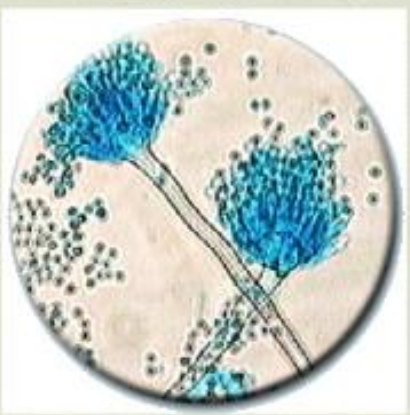
ASPERGILLOSIS

	A. fumigatus	*A. niger*	*A. flavus*	*A. terreus*
Macroscopic morphology	• Velvety or powdery • At first white than turning dark greenish to gray with a narrow white border. • Reverse white to tan	• Wooly • At first white to yellow than turning black • Reverse white to yellow	• Velvety • Yellow to green or brown • Reverse goldish to red brown	• Usually velvety • Cinnamon brown • Reverse white to brown
Picture				

ASPERGILLOSIS

- One of the largest of the fungal genera
- Hundred of species have been recorded
- The most important species:
  - *A. fumigatus*
  - *A. flavus*
  - *A. niger*





CLASSIFICATION OF ASPERGILLOSIS

Airways/nasal exposure to airborne Aspergillus

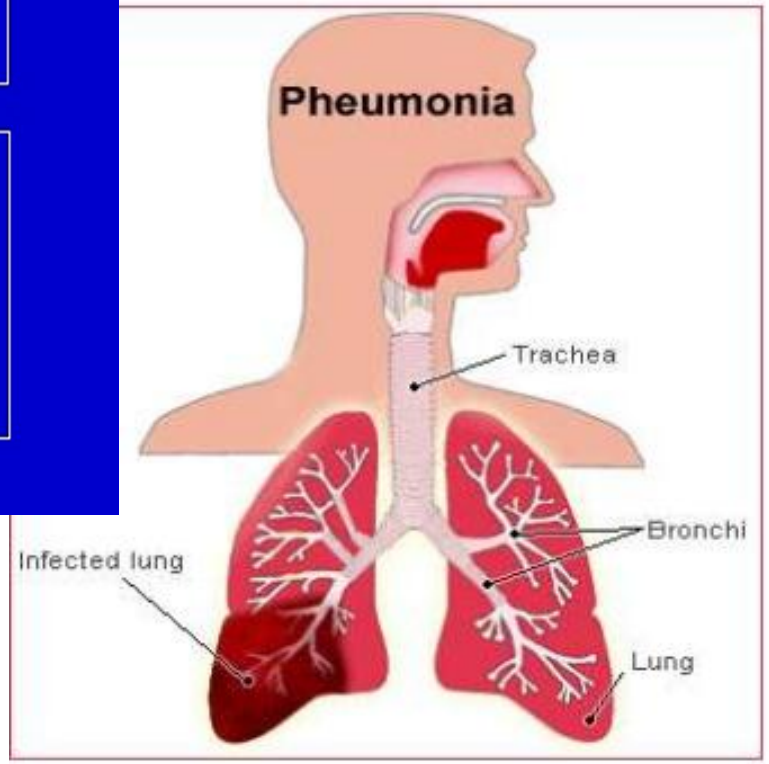
Persistence without disease - colonisation of the airways or nose/sinuses

Invasive aspergillosis
- Acute (<1 month course)
- Subacute/chronic necrotising (1-3 months)

Chronic aspergillosis (>3 months)
- Chronic cavitary pulmonary
- Aspergilloma of lung
- Chronic fibrosing pulmonary
- Chronic invasive sinusitis
- Maxillary (sinus) aspergilloma

Allergic
- Allergic bronchopulmonary (ABPA)
- Extrinsic allergic (broncho)alveolitis (EAA)
- Asthma with fungal sensitisation
- Allergic Aspergillus sinusitis (eosinophilic fungal rhinosinusitis)

Aspergilloma



- Fungus colonize preexisting (Tuberculosis) cavities in the lung and form compact ball of mycelium which is later surrounded by dense fibrous wall presents with cough, sputum production
- Haemoptysis occurs due to invasion of blood vessels
- Cases of aspergilloma rarely become invasive

Laboratory diagnosis

Specimens

Sputum, other respiratory specimens, lung biopsy specimens

Microscopic examination

KOH mount

The fungus appears as non-pigmented septate mycelium, 3-5 µm in diameter with chatracteristic dichotomous branching and an irregular outline

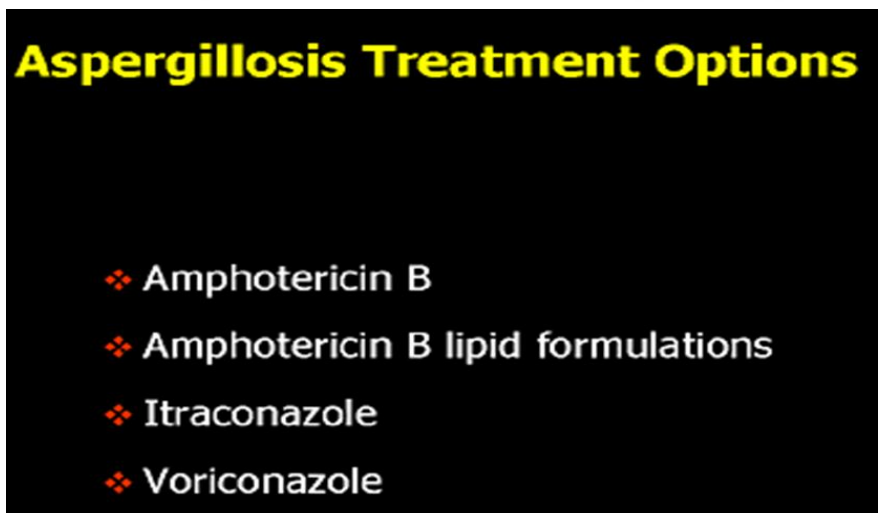
Rarely the characteristic sporing heads of *Aspergillus* species are present

Culture

- Aspergillus species grow readily on SDA without cycloheximide at 25-37^{0}C
- Colonies appear after 1-2 days of incubation
- Species are identified according to the morphology of their conidial structures

Skin tests

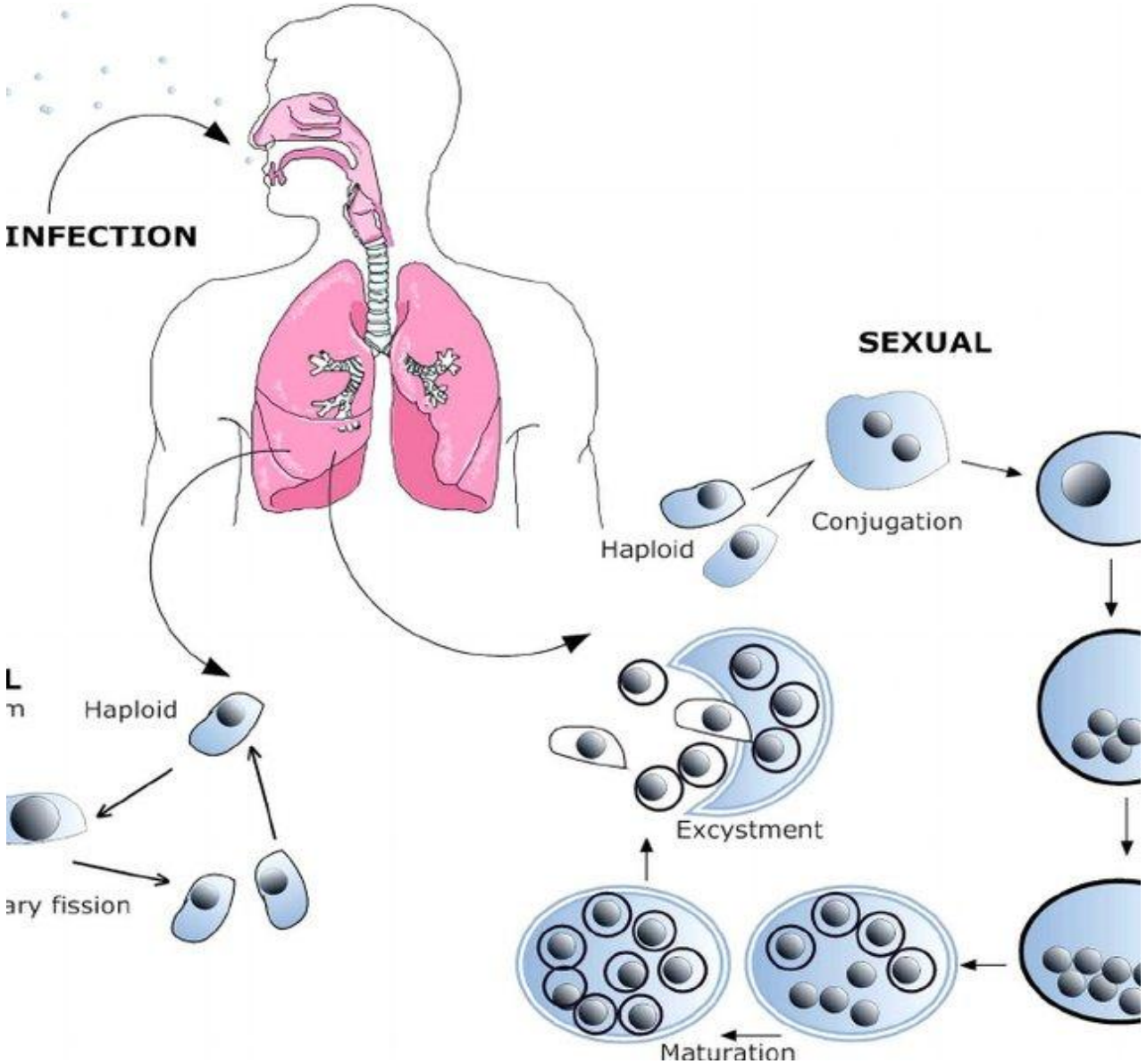
- Skin tests with *Aspergillus* species antigen are useful for the diagnosis of allergic broncho pulmonary aspergillosis

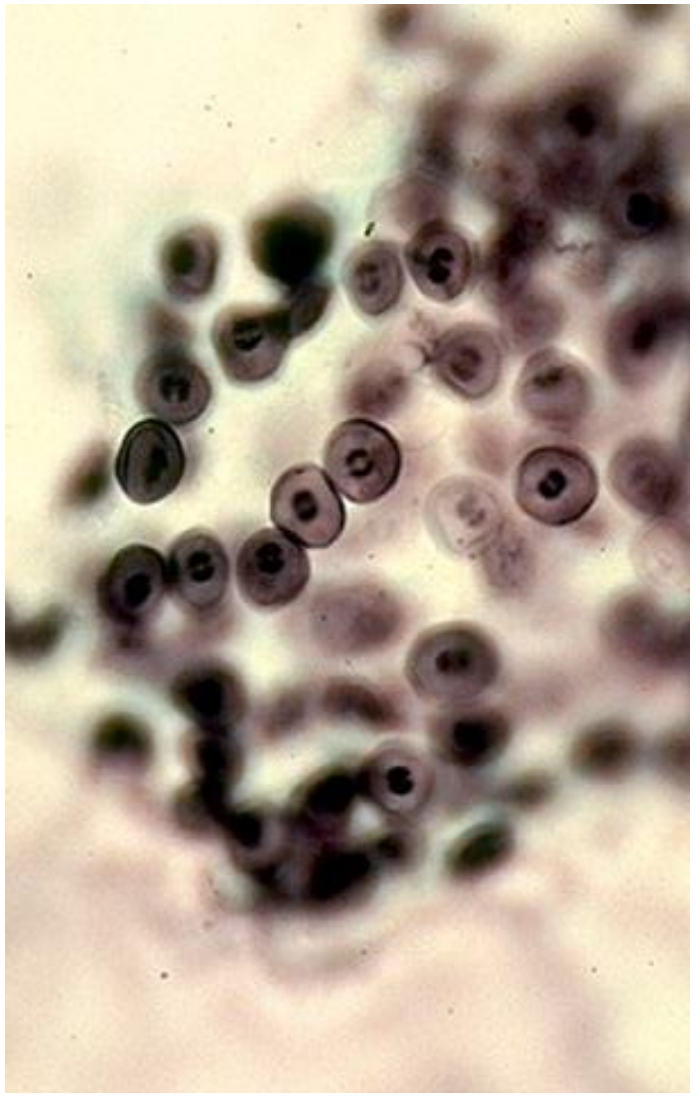


Pneumocystis jiroveci

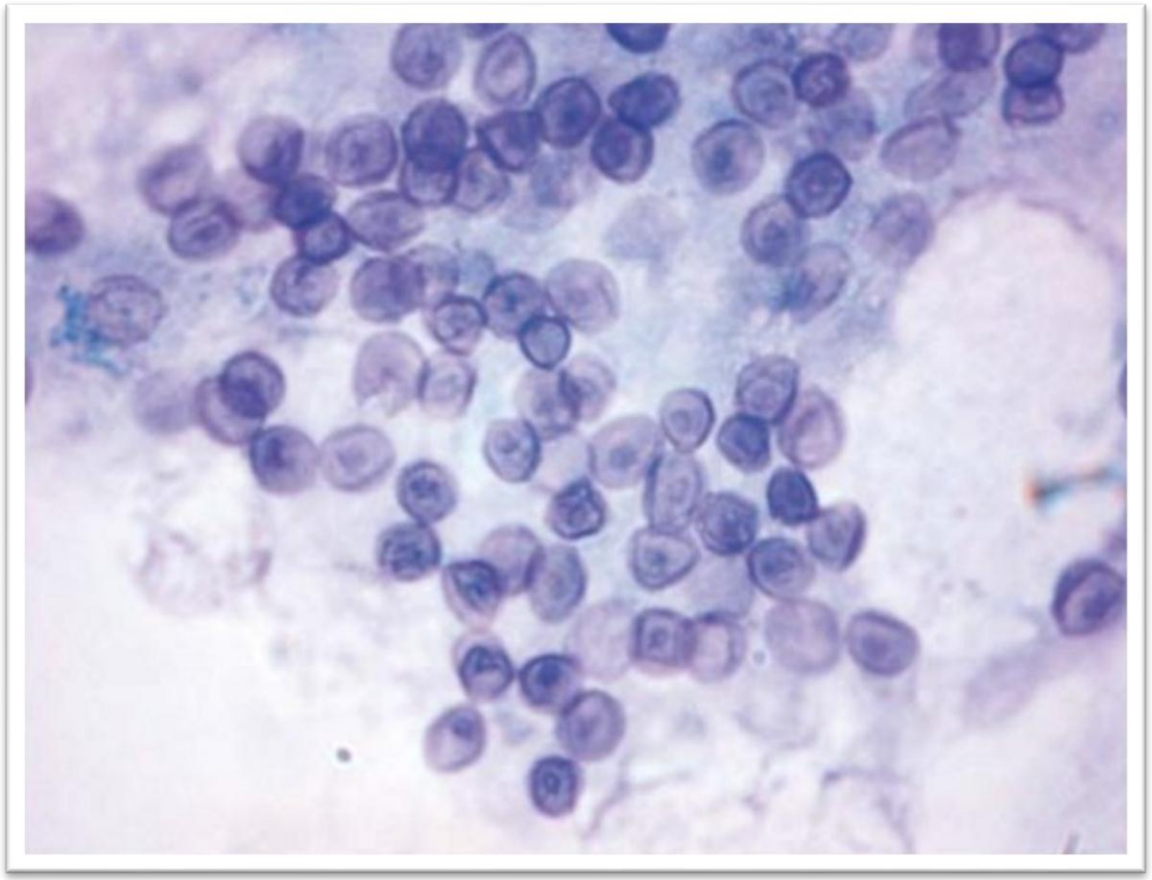
- *Pneumocystis jiroveci* causes pneumonia in immunocompromised individuals.
- Microorganisms of the genus Pneumocystis were previously considered protozoa. However, molecular-biological and genetic studies have shown that it is an ascomycete-like fungus.
- However, they are similar to protozoa in morphology and other properties, sensitivity to antimicrobial drugs, and it is possible that these are the transitional microorganisms between protozoa and fungi.

Pneumocystis jiroveci





Pneumocystis jiroveci



Pathogenetic features of pneumocystosis pneumonia

In cases of immunodeficiency other than AIDS, interstitial pneumonitis with plasmatic cells develops as a result of infiltration of the intercellular areas of the alveoli with plasmatic cells.

In pneumocystosis pneumonia observed during AIDS, there are no plasmatic cells, the blockade of gas exchange in alveoli causes respiratory failure.

P.jiroveci is an extracellular parasite. In the lung tissue, the extracellular parasite aggregates tightly to the alveolar epithelium, and their growth is usually limited by the surfactant layer on the surface of the alveolar epithelium.

P.jiroveci does not cause disease in people without immunodeficiency

Microbiological diagnostics

- It is possible to detect trophozoites and cyst forms in smears prepared from bronchial lavage and sputum, stained with Giemsa, silvering, as well as with toluidine.
- In smears stained by the Giemsa method, the cytoplasm of the fungus is blue, and the nucleus is red-purple.
- It is also possible to detect the originators in patches by means of IFR.
- P.jirovecii is a non-cultivable microorganism, so the cultural method is not used.
- P.jirovecii is part of the obligate microflora of the human body, so the detection of antibodies in the blood serum is not of diagnostic importance.
- Treatment is carried out with trimethoprim sulfometaxazol (biseptol) and pentamidine.

The causative agents of mycotoxicoses

- Many mold fungi distributed in soil and plants synthesize toxic substances - mycotoxins. Mycotoxins are secondary metabolites of these fungi and are thermostable substances. Their accumulation in food products contaminated with mold fungi causes food poisoning in humans and animals - ***mycotoxicoses.*** The producers of mycotoxins are mainly grains, rice, corn, etc. Although it contaminates cereals, it can also be found in other food products. Mycotoxins accumulate during harvesting, storage and processing of agricultural plants and food products under unfavorable conditions.

Forms of mycotoxicoses:

- ***Aflatoxicosis*** is a mycotoxicosis caused by aflatoxins mainly synthesized by *Aspergillus flavus.* Aflatoxins are widely distributed in plant foods. Aflatoxins do not decompose during thermal processing and have high toxicity. Acute poisoning caused by aflatoxins in animals is characterized by slowness of movement, convulsions, paresis, hemorrhages, edema, and liver damage, and has high lethality. Necrosis, cirrhosis, and primary cancer develop in the liver.

Forms of mycotoxicoses:

- ***Fusariotoxicoses*** are mycotoxicoses caused by mycotoxins of *Fusarium* fungi. The development of the *Fusarium sporotrichiella* fungus on cereals causes the accumulation of mycotoxins in them. the use of such cereals in food causes mycotoxicosis - alimentary-toxic alekia. As a result, the amount of granulocytes in the blood decreases sharply, and then acute damage to myeloid and lymphoid tissues, bone marrow necrosis develops, which leads to a violation of hematopoiesis.
- The mycotoxin of the *Fusarium graminearum* fungus has a neurotropic effect and causes mycotoxicosis - ***"drunk bread"*** syndrome with symptoms such as weakness, impaired gait, acute headaches, dizziness, vomiting, diarrhea, abdominal pain.

Forms of mycotoxicoses:

- ***Ergotism*** occurs when cereal crops, mainly rye, are damaged by the fungi Clavuiceps purpurea and Clavuiceps paspalum. Mycotoxins of ryegrass consist of alkaloids of lysergic acid and clavine alkaloids, which have neurotoxic effects.
- The acute form of ergotism is accompanied by gastroenteritis and neurological symptoms - paresthesias and convulsions, often resulting in death. The chronic form is accompanied by symptoms of polyneuritis, vomiting, gastrointestinal disturbances.

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

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.

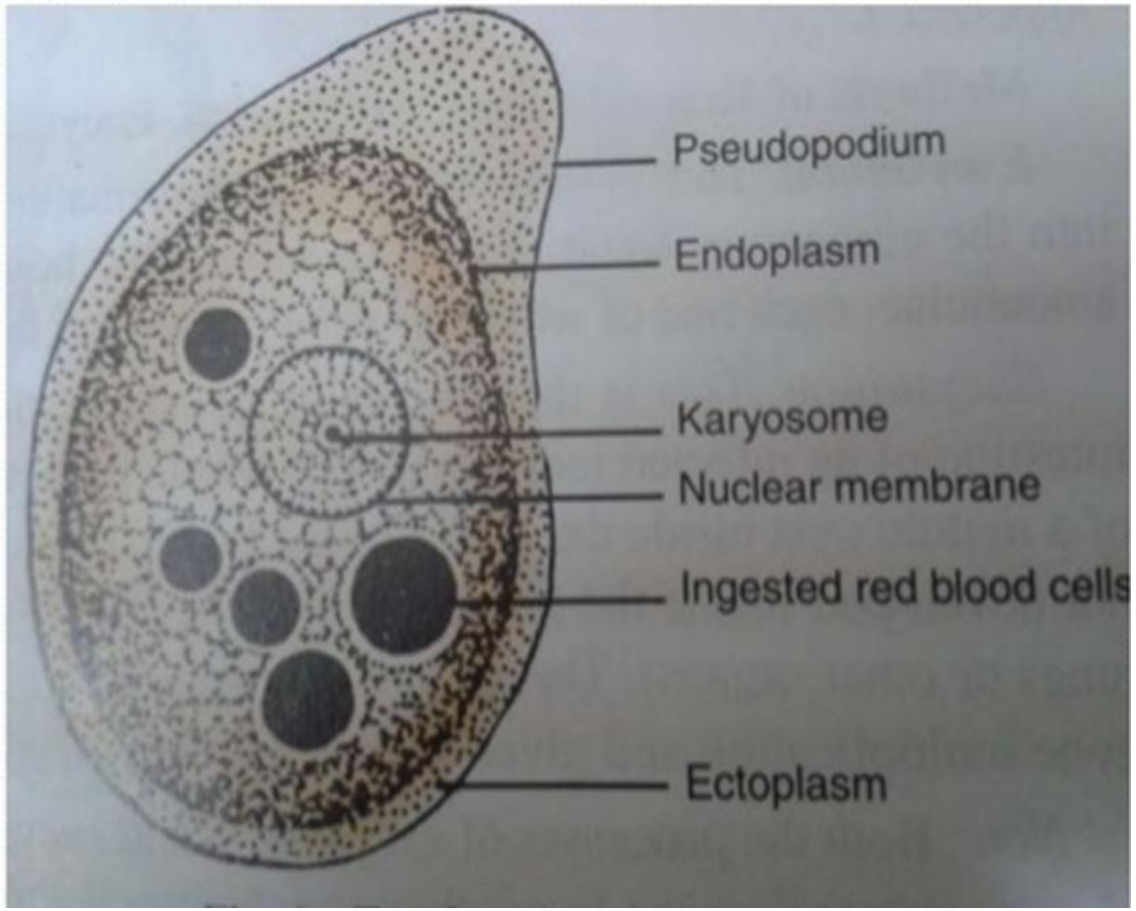
Size: Average being 20-30 um.

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.

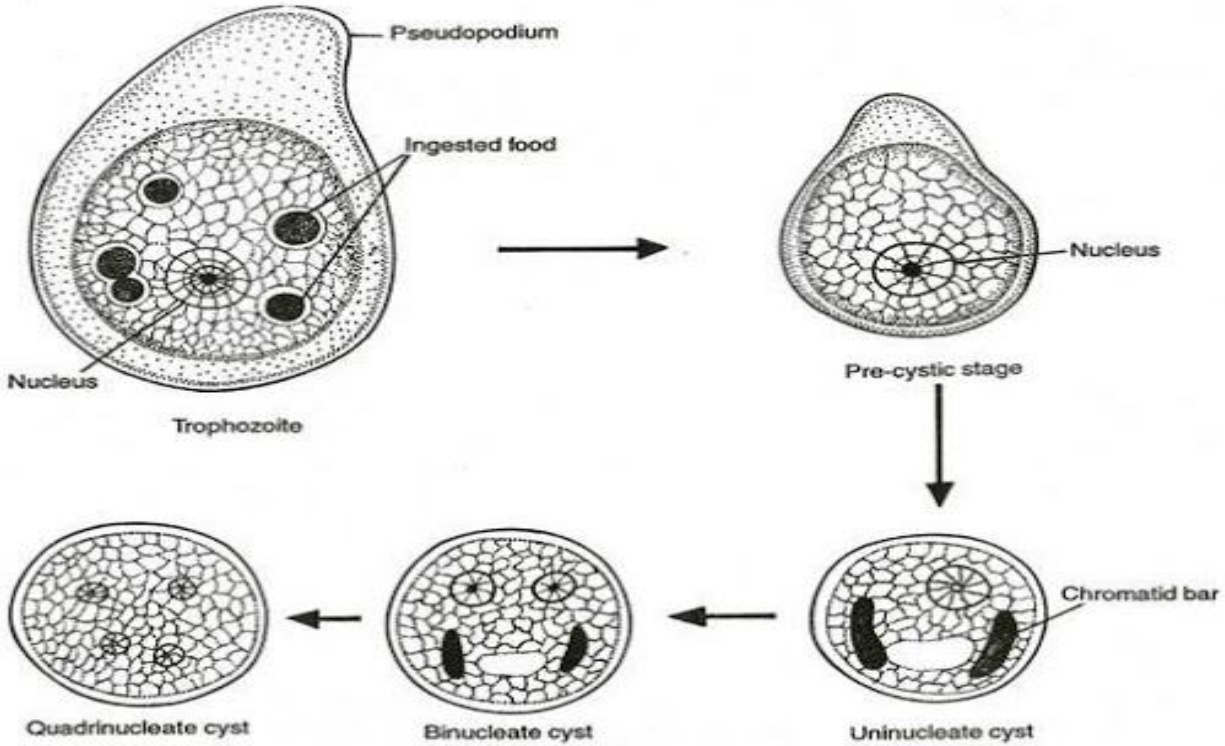
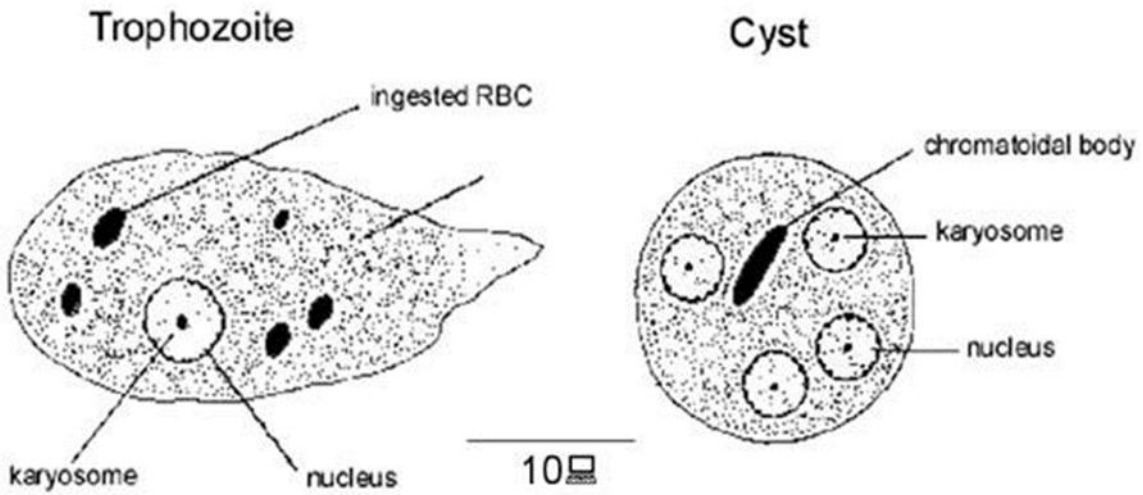


Fig. 174. Stages of life cycle of *Entamoeba histolytica*.



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.**

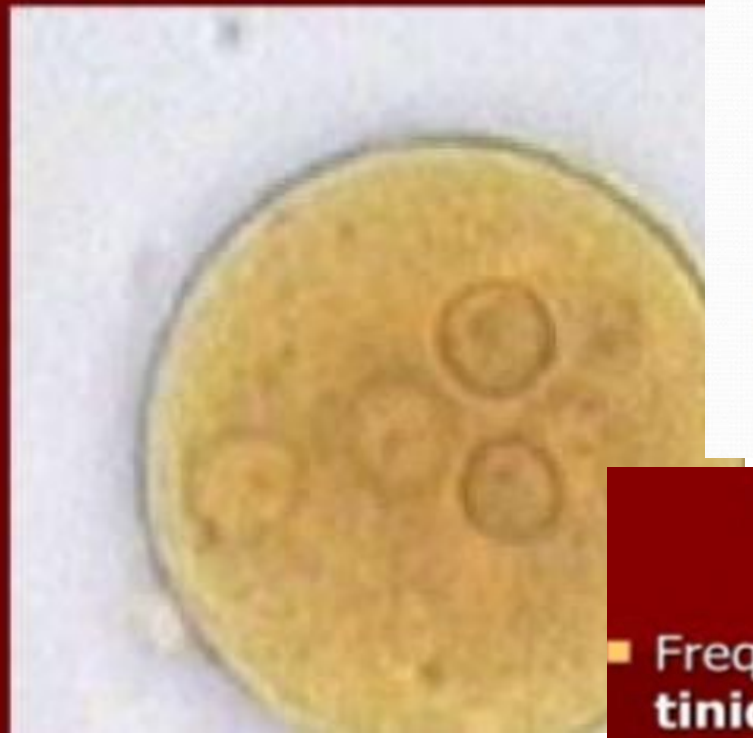
1.Examination of Stool:

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.

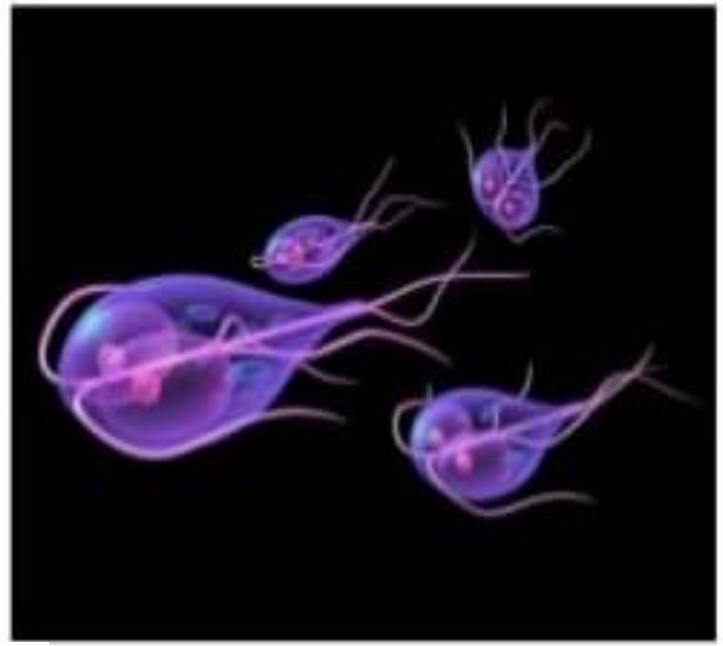
ELISA, Indirect haemagglutination, Dot ELISA, latex agglutination test are in use.

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.

MORPHOLOGY

- It exists in two forms –

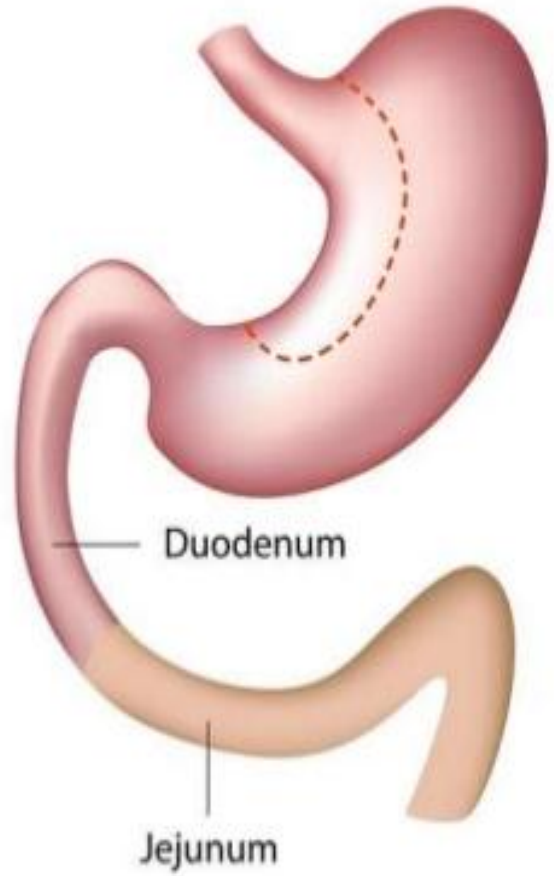
1) Trophozoit (Vegetative form)

2) Cyst (Infective form)

HABITAT

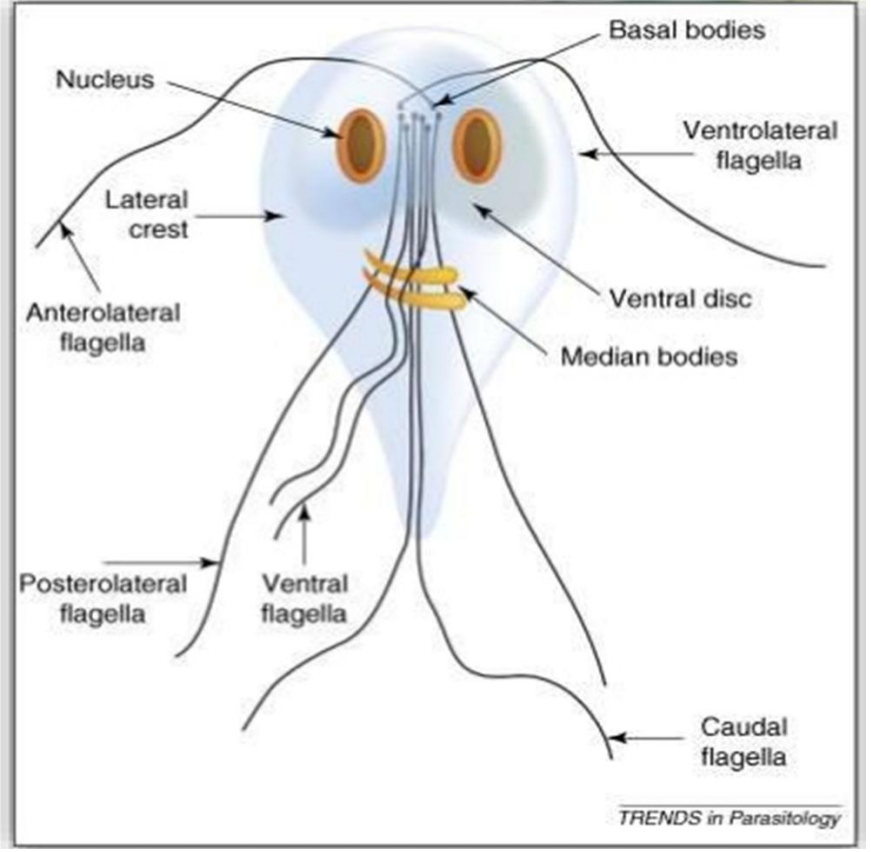
- Duodenum & the upper part of the jejunum.

- THE ONLY PROTOZOAN PARASITE FOUND IN THE LUMEN OF HUMAN SMALL INTESTINE.

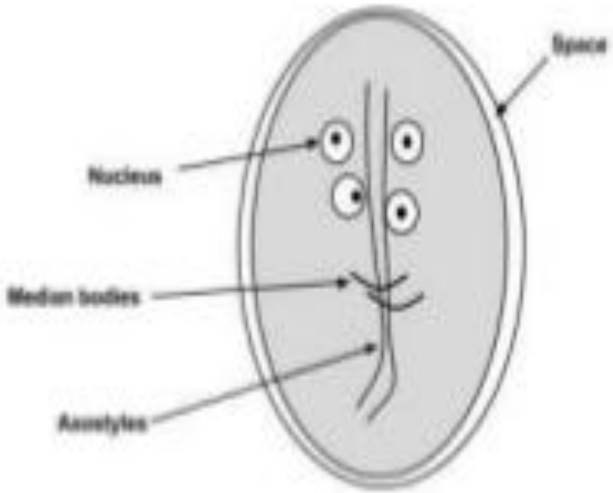


TROPHOZOITE

- 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.



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

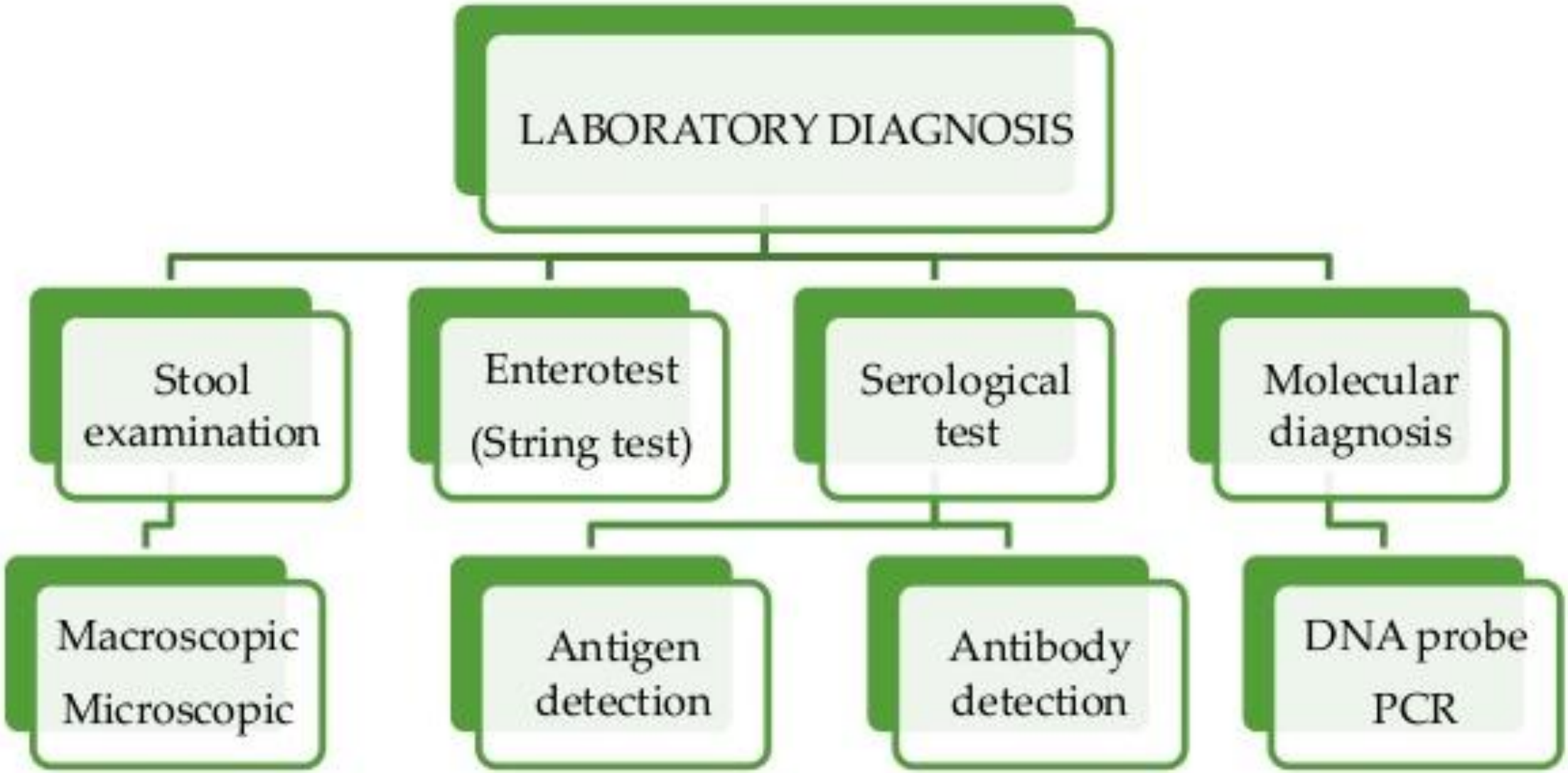
- Infection is occured by ingestion of cyst in contaminated food & water.

- 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

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**

Balantidium coli

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

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

MORPHOLOGY

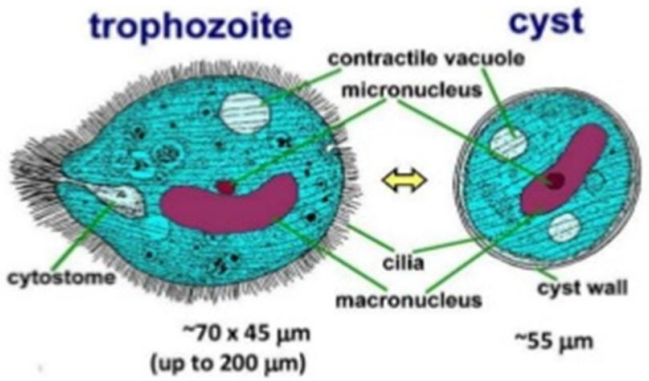
- Two forms:

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

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

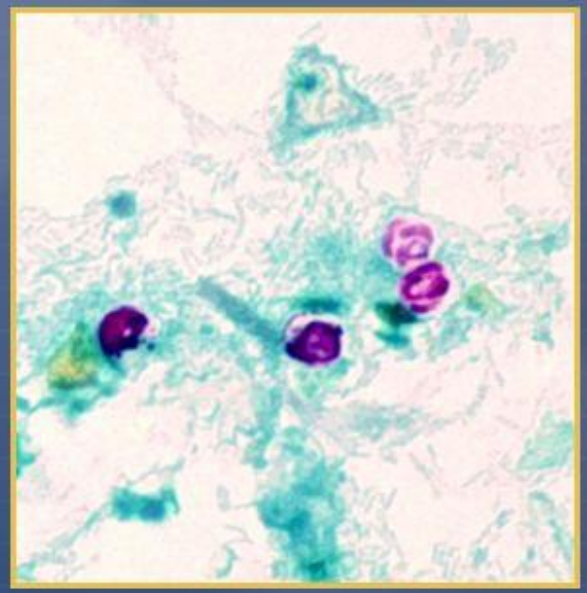
- Microscopically- cluster of trophozoites are found in sub mucosa with inflammatory cells (lymphocytic)
- Patients present have frequent diarrhoea with profuse mucus and blood.
- Other features- fever, nausea, vomiting and abdominal pain
- Haemorrhage- may lead to shock and death

LABORATORY DIAGNOSIS

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

Cryptosporidium parvum

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



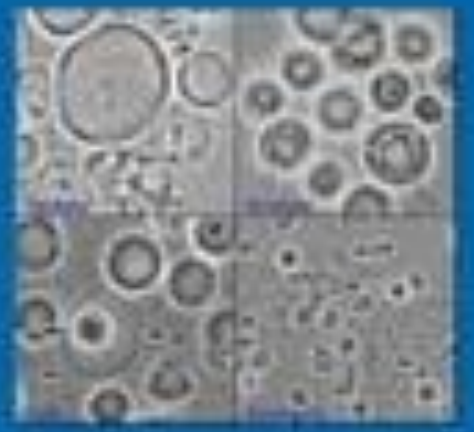
Oocysts

- 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

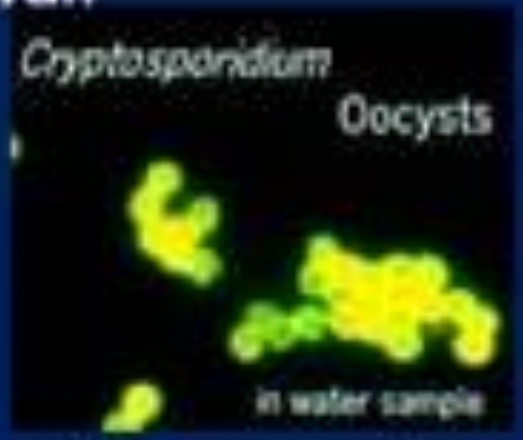
- Excystation -release of the four sporozoites
- Invasion of intestinal epithelial cells
- Asexual life cycle
- Sexual life cycle
  - Differentiation of micro and macrogametes
- Development of oocysts
- Formation of new, infectious sporozoites

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

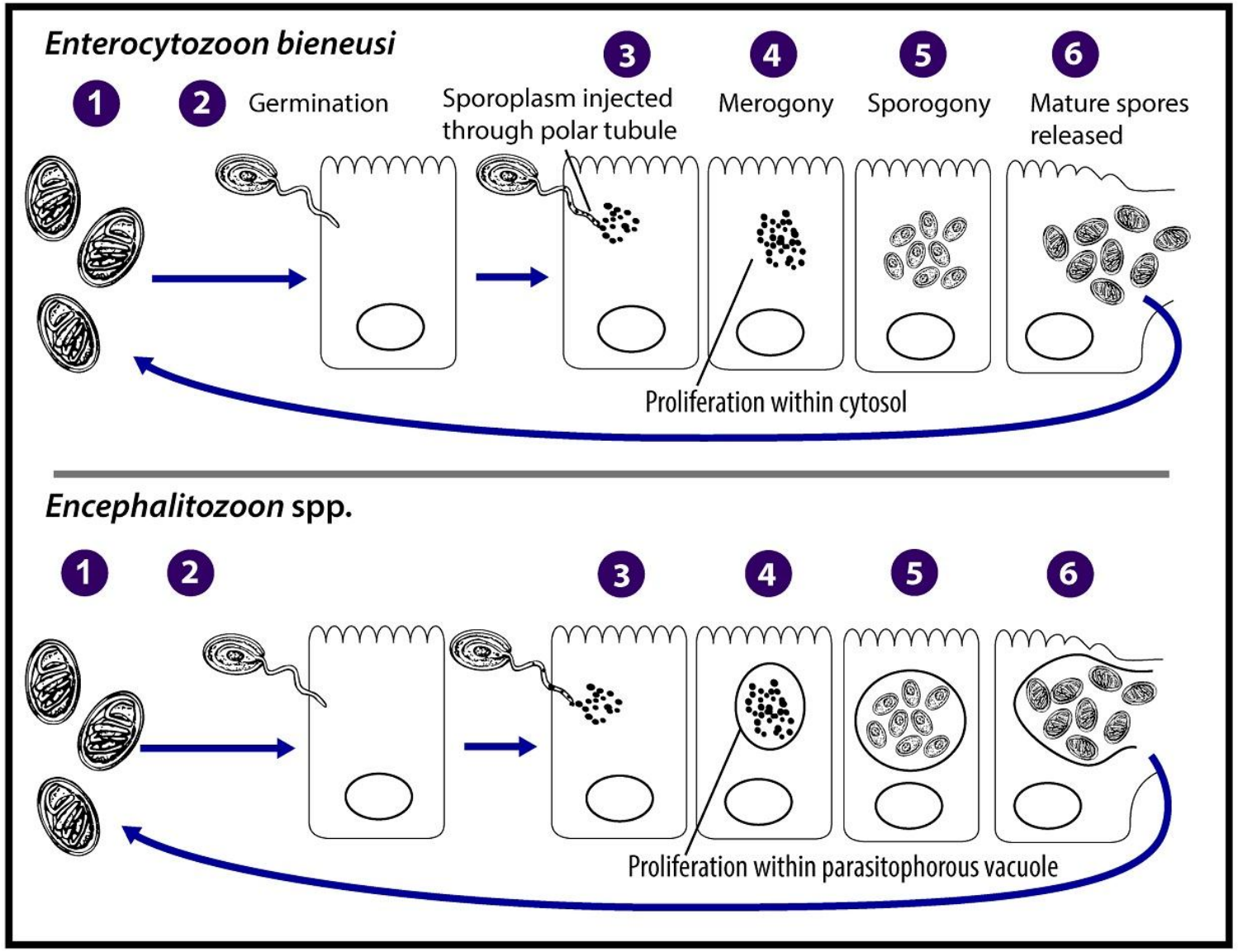
- To date, more than 1400 species belonging to over 200 genera have been described as parasites infecting a wide range of vertebrate and invertebrate hosts. There are at least 15 microsporidian species that have been identified as human pathogens; the vast majority of cases being caused by ***Enterocytozoon 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***.

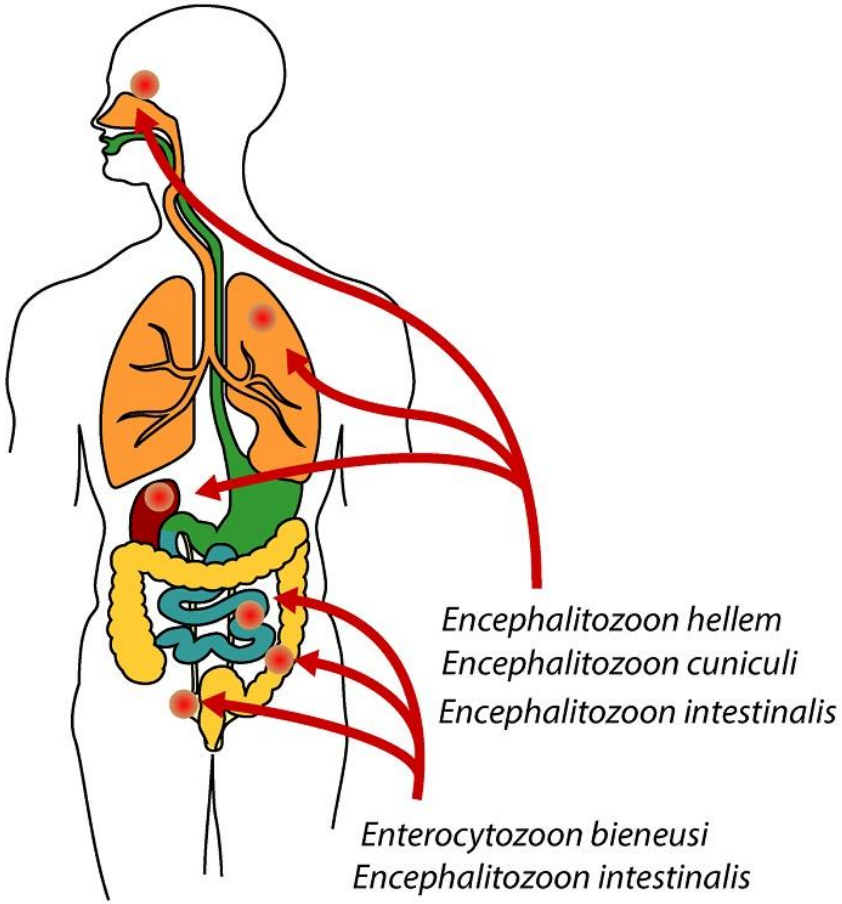
Life Cycle

- 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

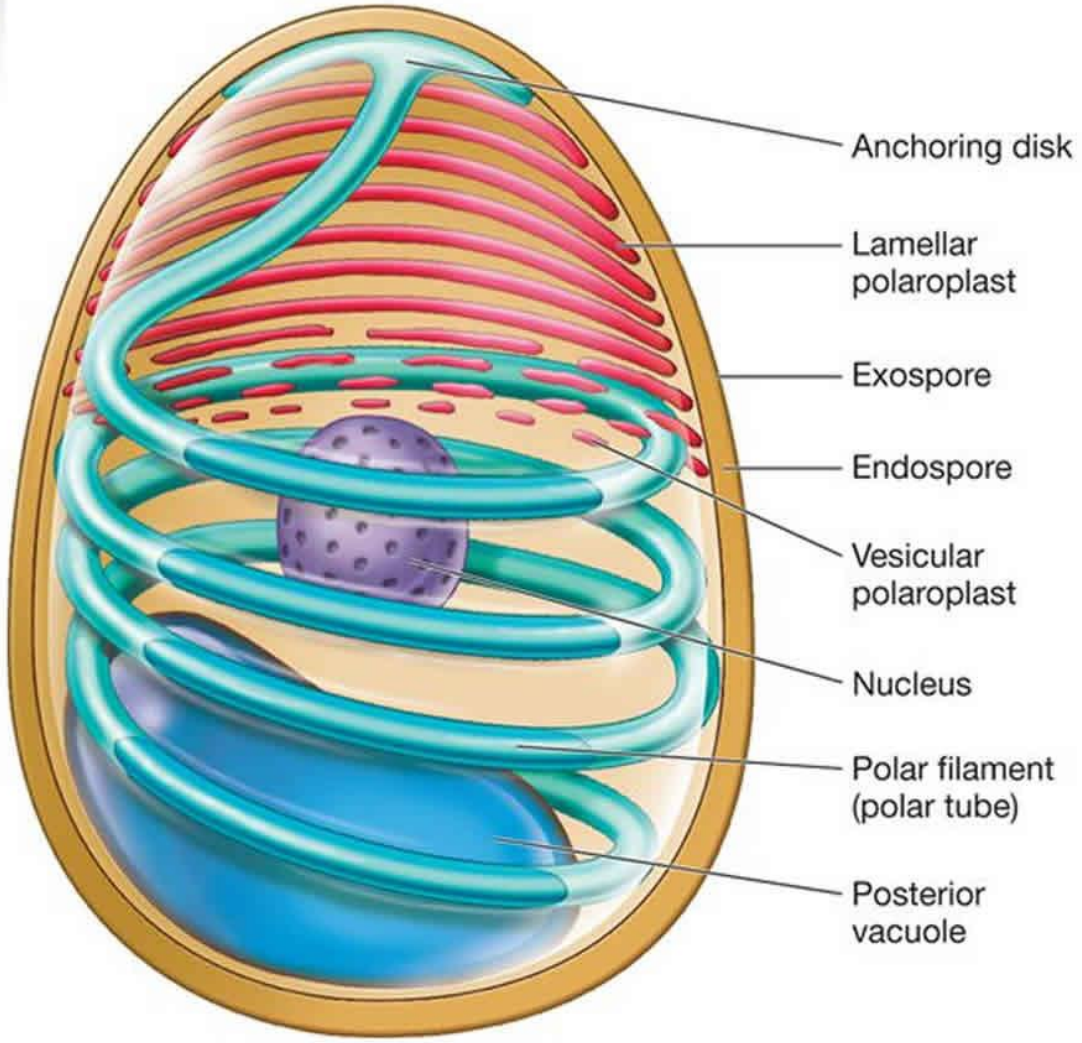




Clinical Presentation

- 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.

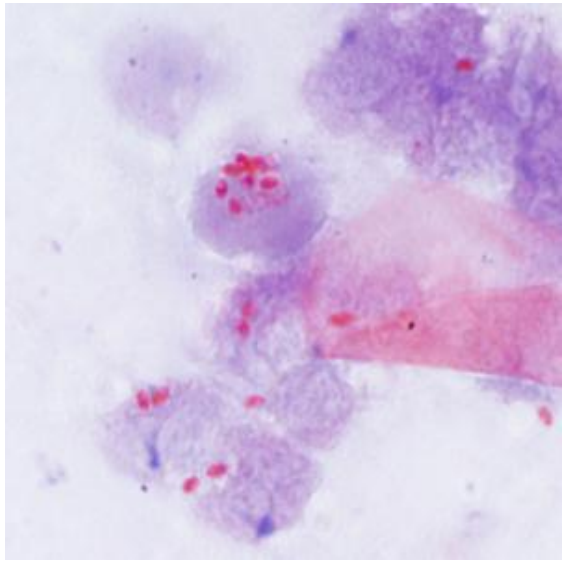
•Immunofluorescence Assays (IFA)

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

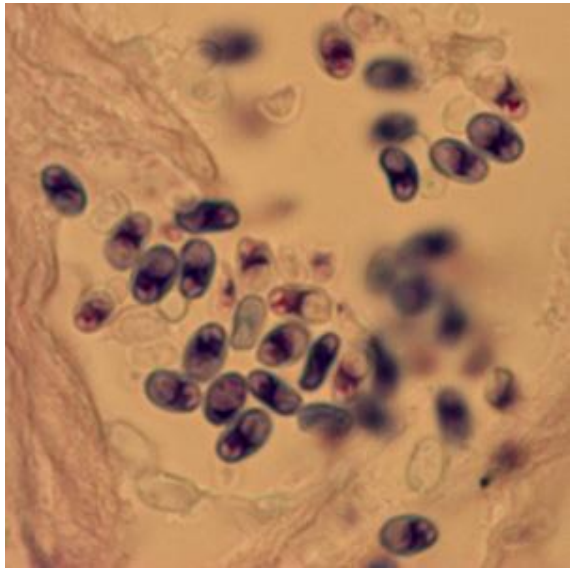
•Molecular Methods (PCR)

•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.

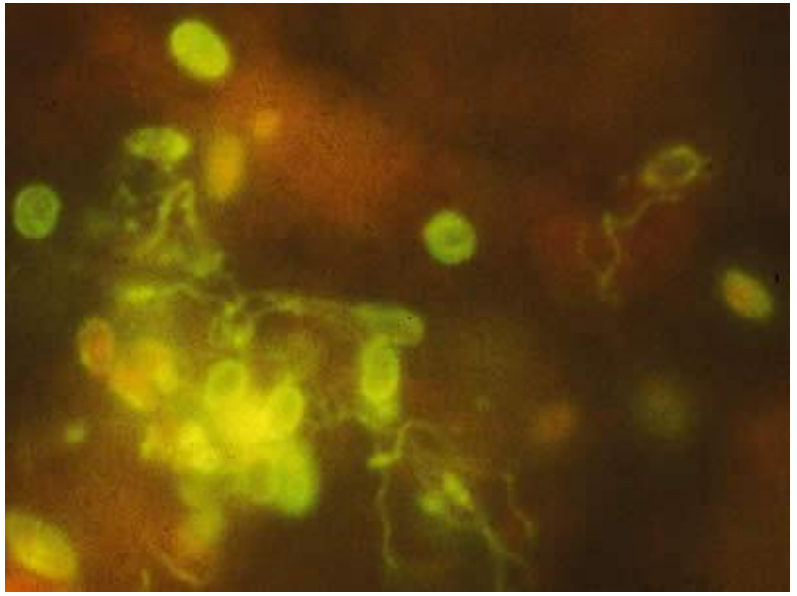
Laboratory diagnosis



Trichrome blue



Giemsa



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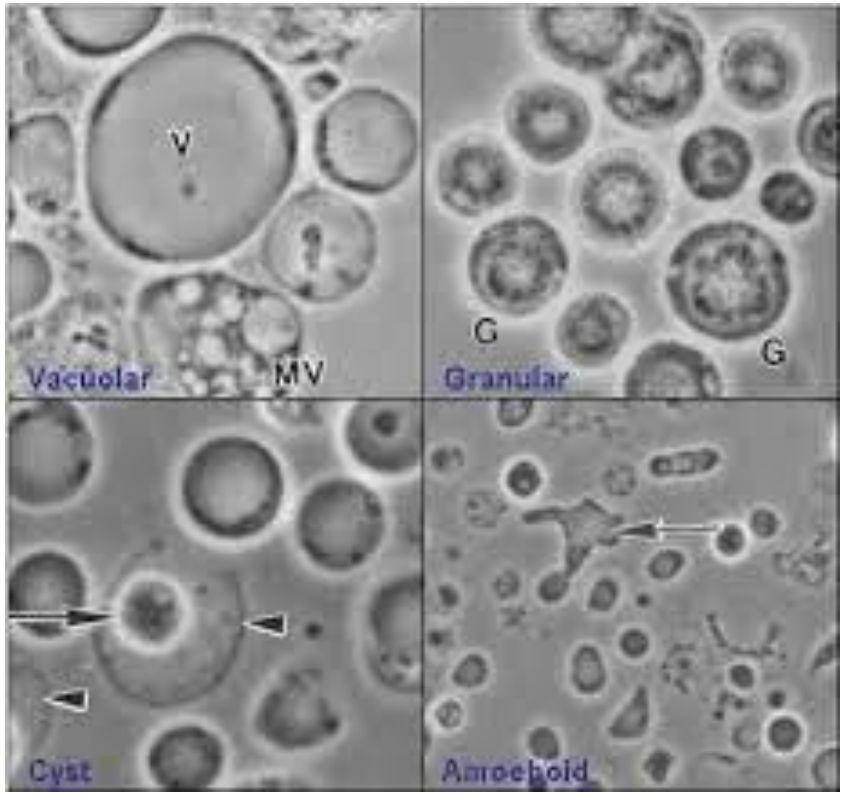
Blastocystis sp.

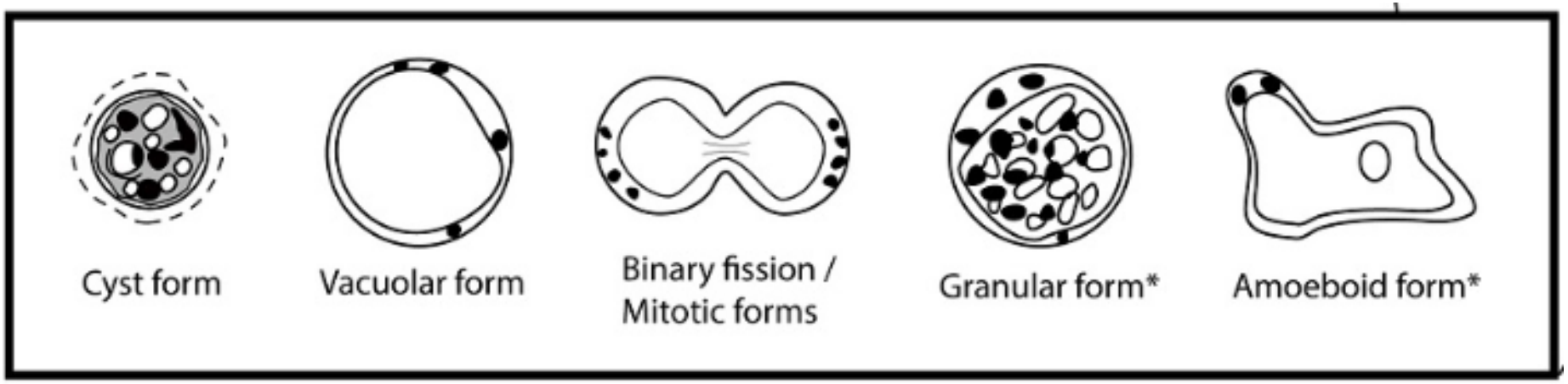
The genus Blastocystis belongs to the order Blastocystida of the class Blastocystea of the phylum Bygira. Previously, blastocysts were thought to be yeast fungi. Currently, they are considered protozoa.

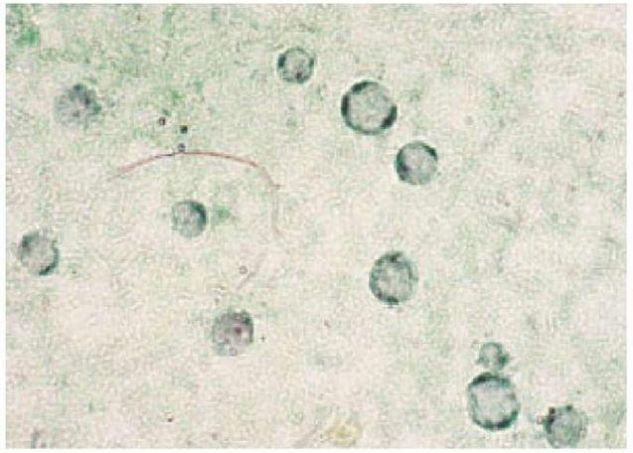


- (Kingdom): Chromista
- **(Phylum):**Bigyra
- (Class): **Blastocystea**
- (Order): **Blastocystida**
- (Family): **Blastocystidae**
- (Genus): ***Blastocystis***
- ***(Species): Blastocystis*** hominis

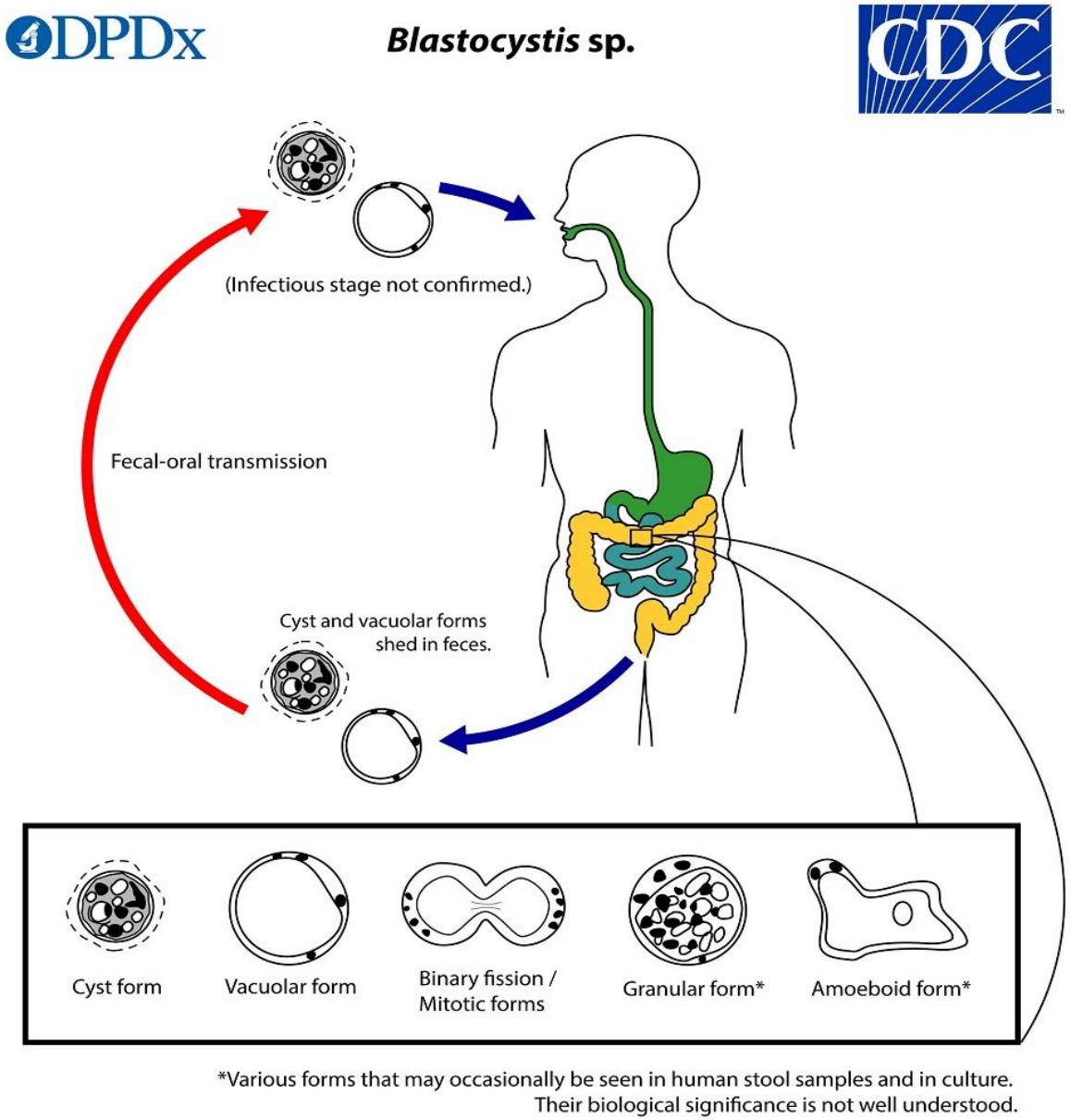
Blastocystis sp.

- 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

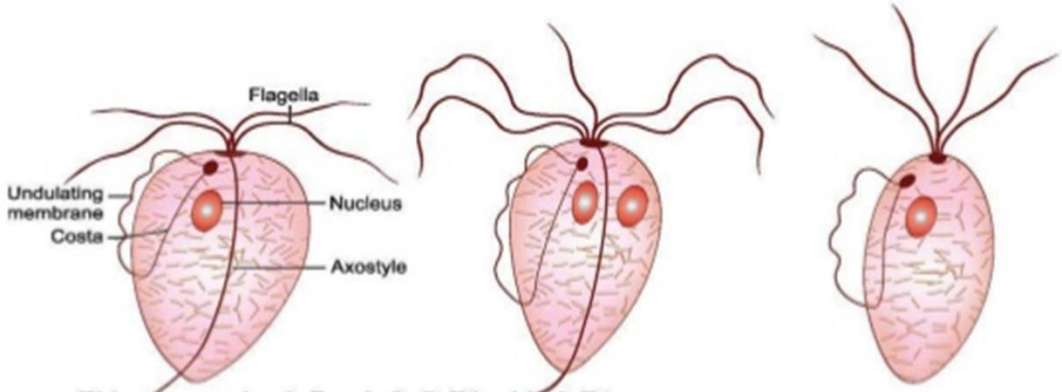
- Characteristically have a cytostome,
- An anterior tuft of flagella,
- Undulating membrane with the recurrent flagellum,
- An axostyle protruding through the posterior end ,
- Only trophozoite stage.

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.

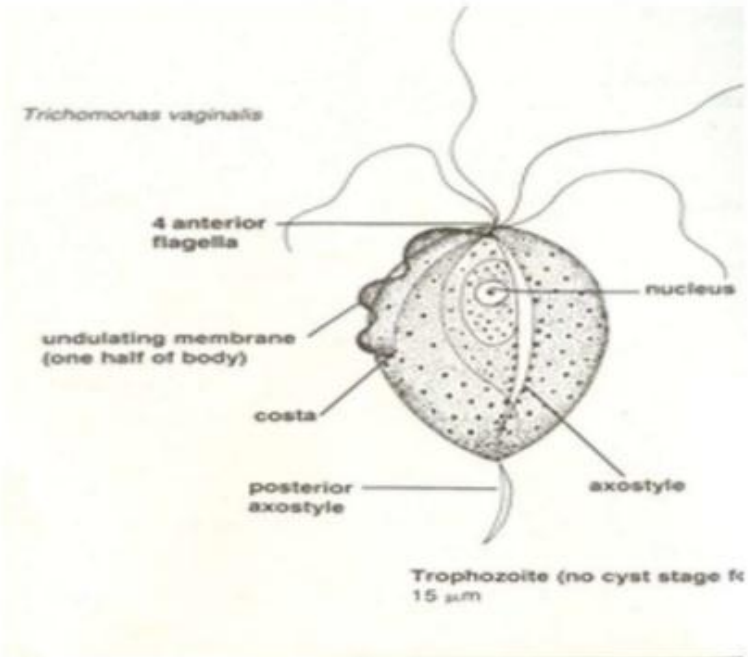
- Fifth flagellum curve back along the margin of the undulating membrane and is called the recurrent flagellum.

- 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).

WOMEN (SYMPTOMATIC)

- Vulvo vaginitis (Trichomonal vaginitis)
- Urethritis

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.

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.

LAB DIAGNOSIS

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

SPECIMENS

- **IN WOMEN :** vaginal discharge, endocervical specimens.
- **IN MEN:** Prostatic fluid, less commonly semen.
- **common specimens** urethral swab, early morning first voided urine sediment.

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

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

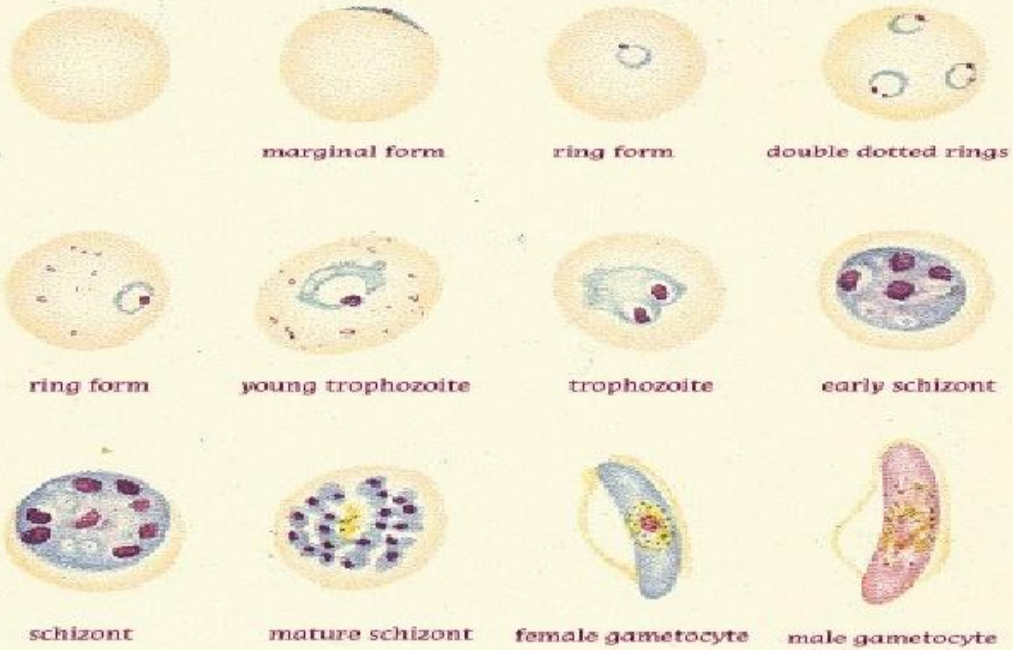
Suborder:- Hemosporidia

Genus:- Plasmodium

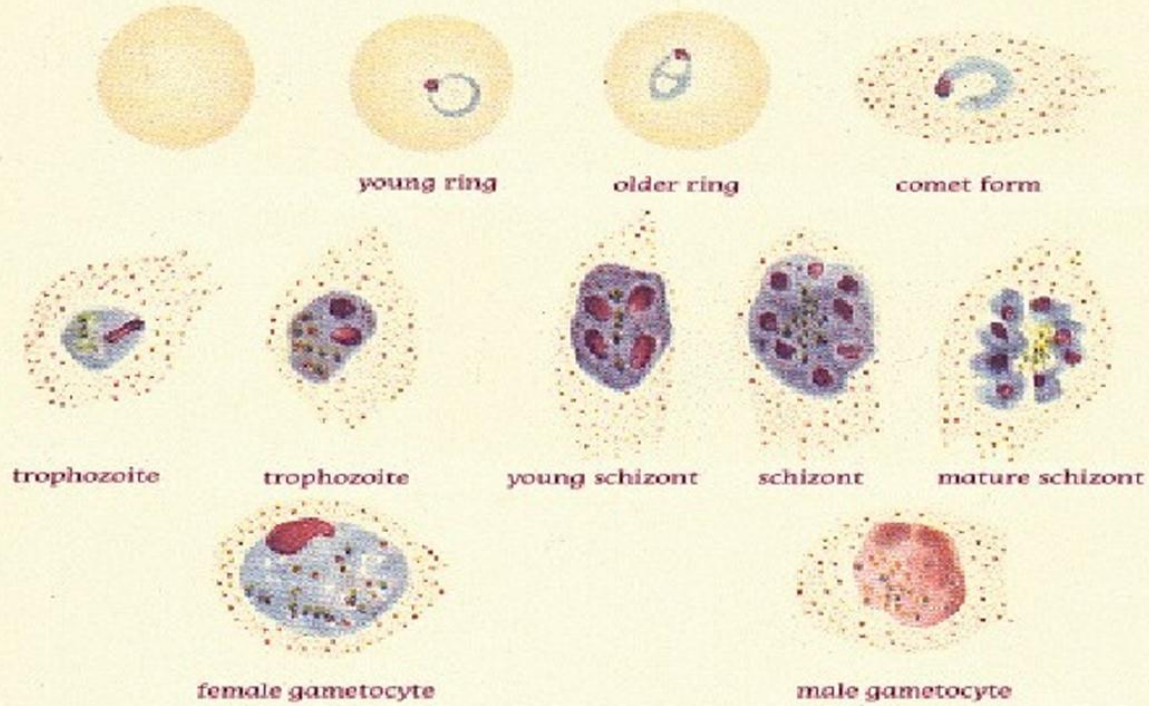
Most common species found in man are :-

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

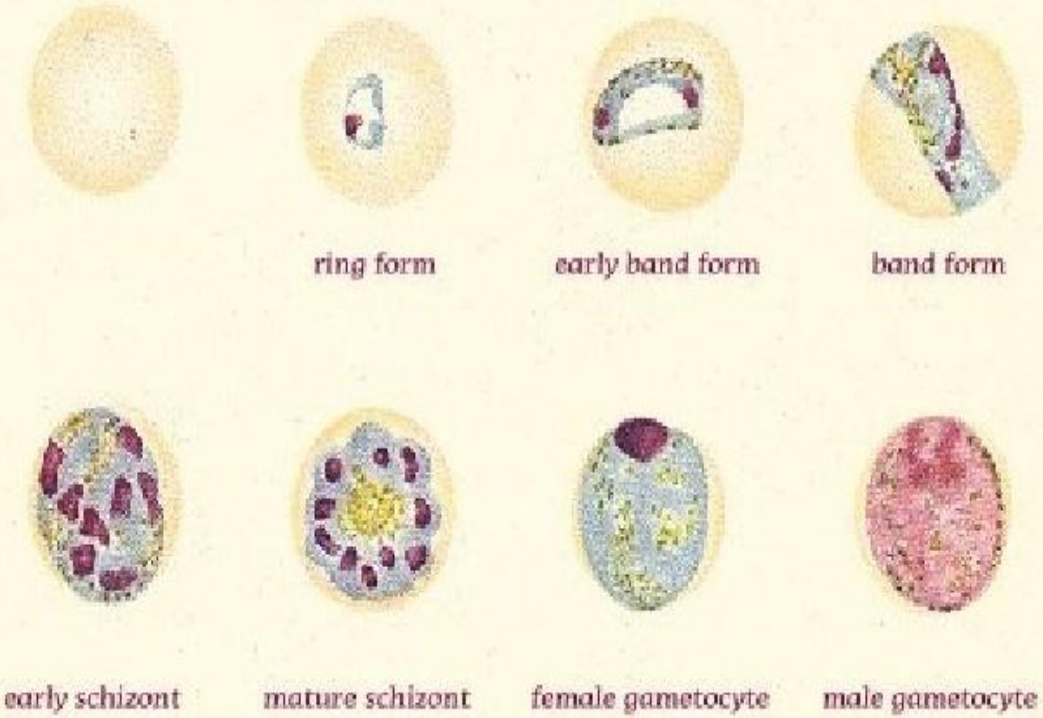
P. falciparum



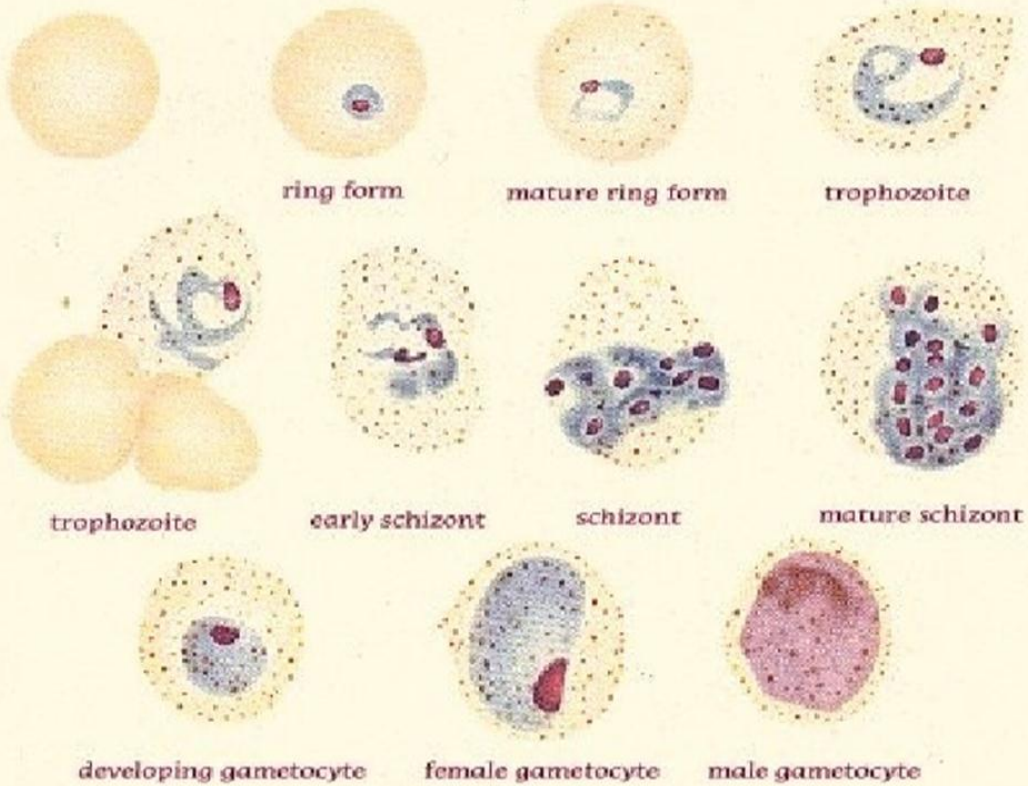
P. ovale



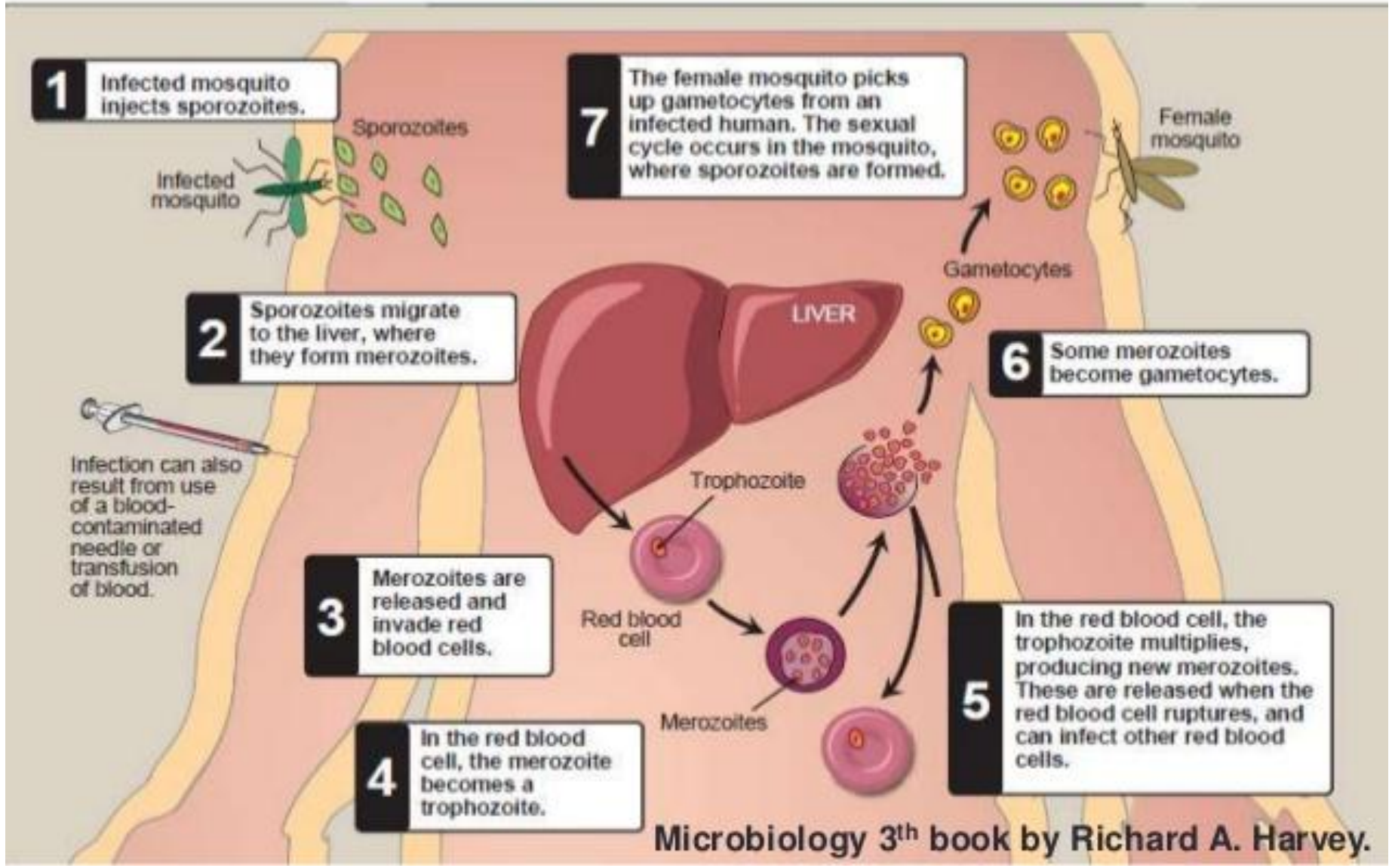
P. malariae



P. vivax



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.

- 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 6o 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**

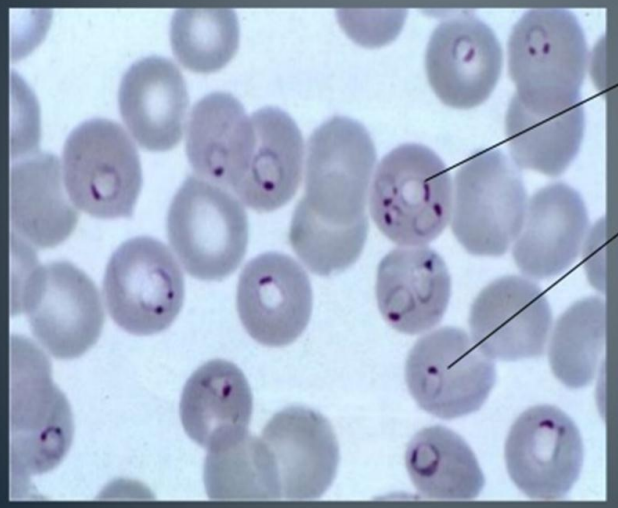
Signs and symptoms of malaria

- 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

Appearance in blood film in Microscope



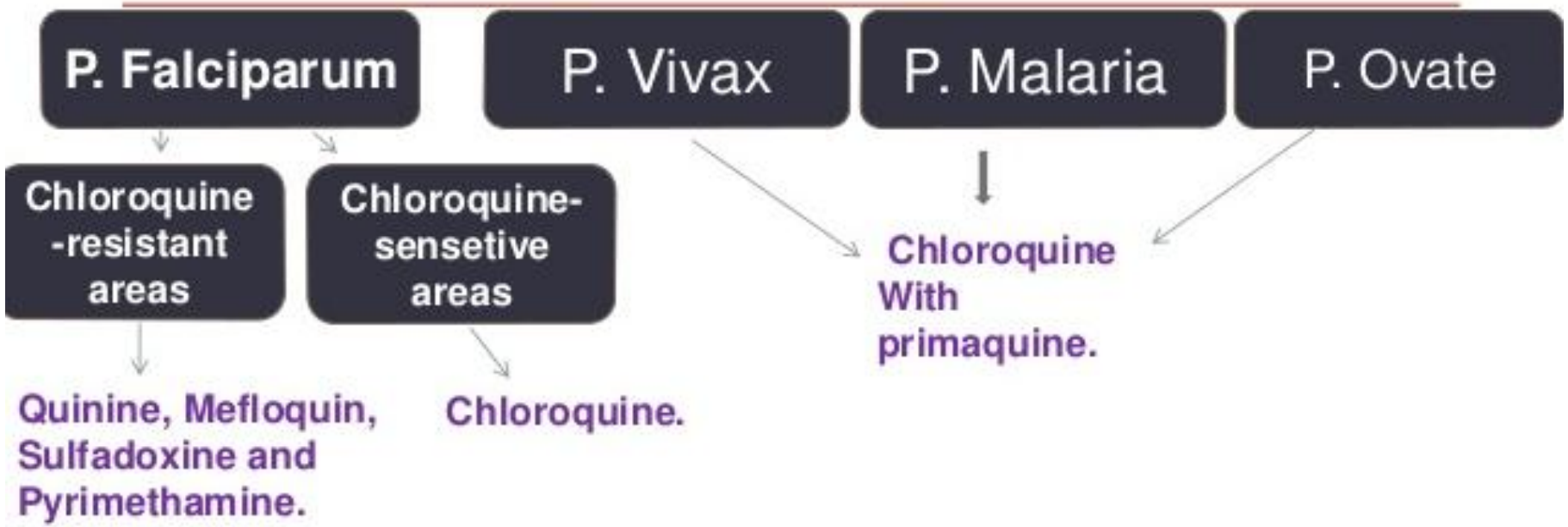
Ring forms or trophozoites; many

red cells infected –
some with more than one parasite

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

Morphology:

Three morphological forms

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

- 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

Image of a tachyzoite

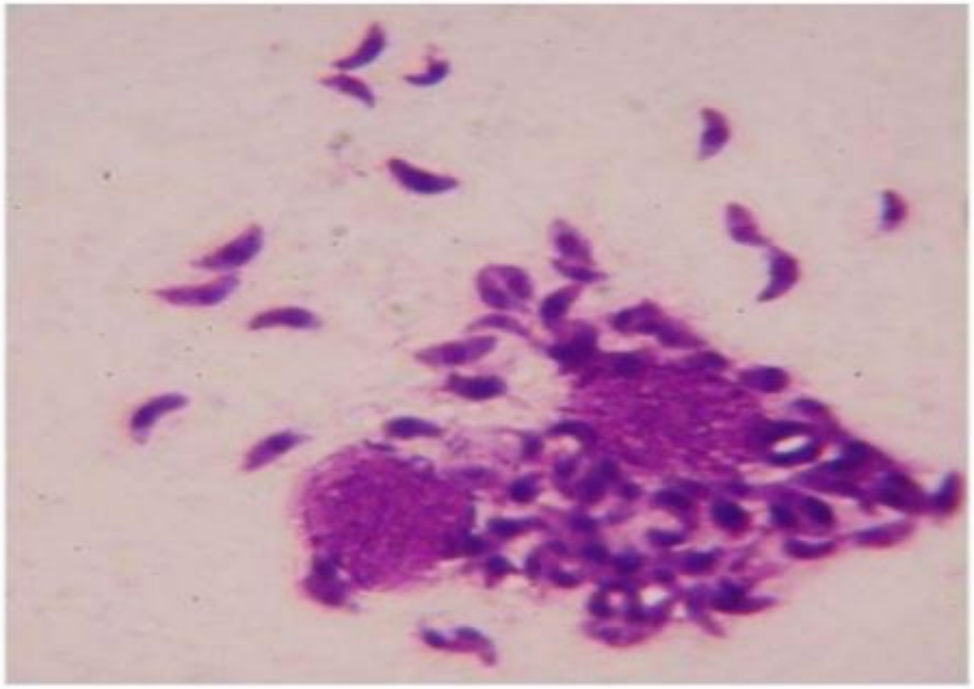
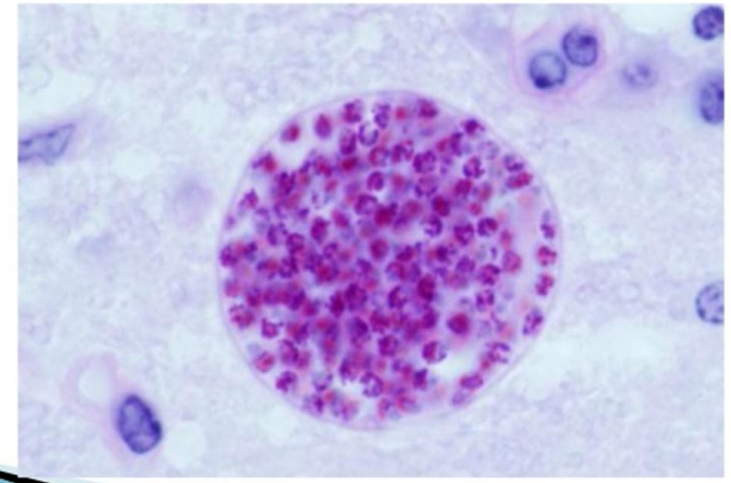
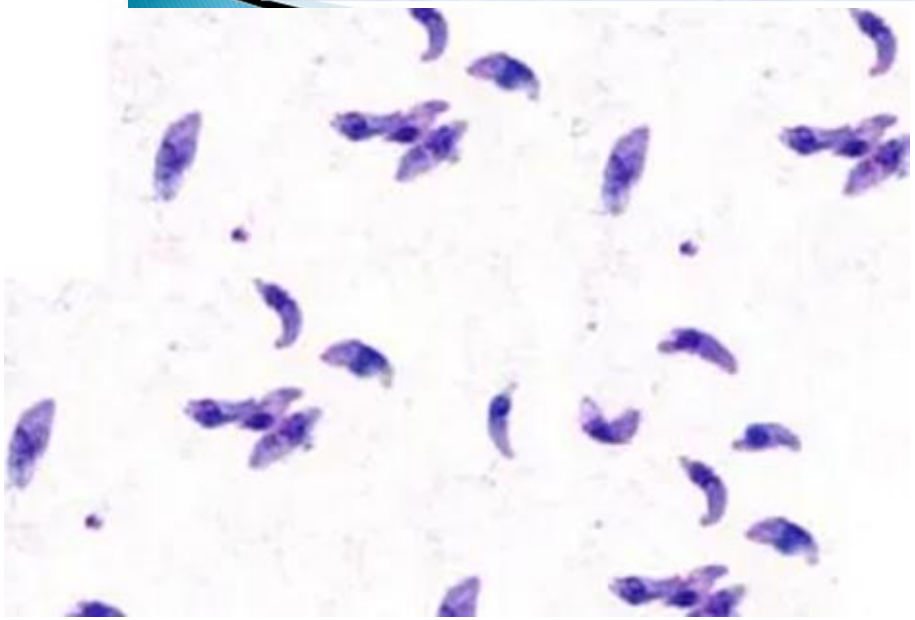


Image of a tissue cyst:





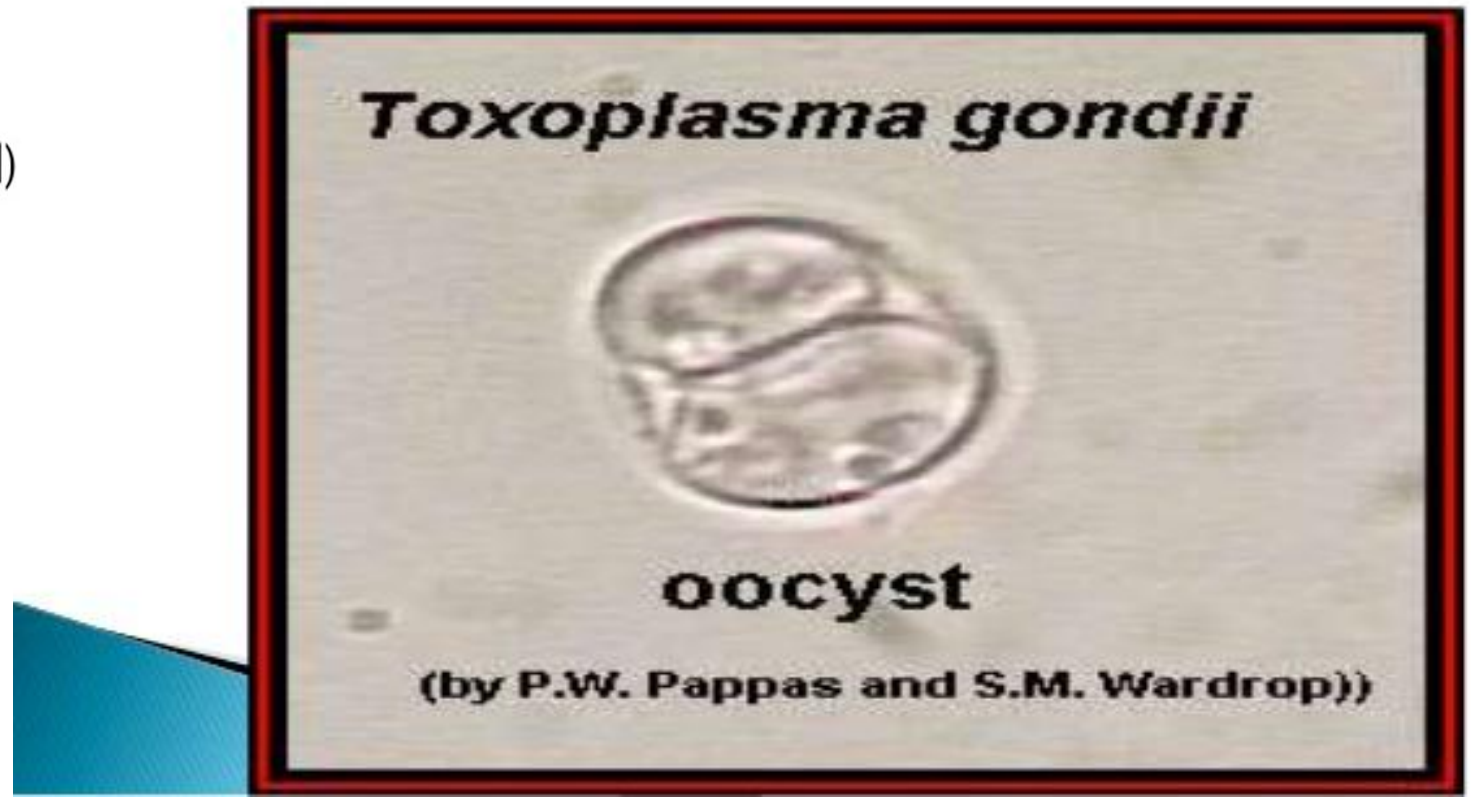
Transmission to man:

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)

OOCYST:

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



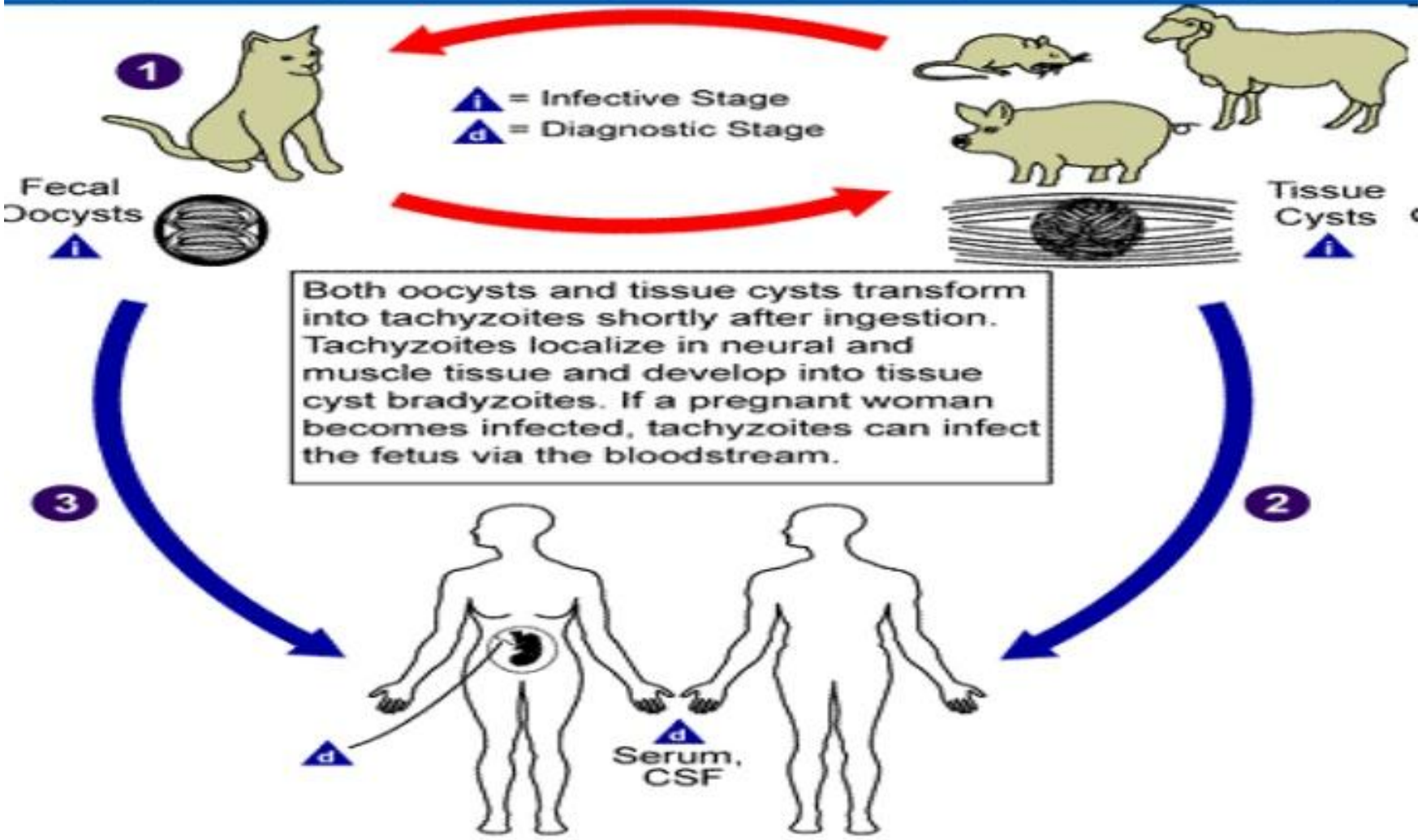
Toxoplasmosis

- 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

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

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

- 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.

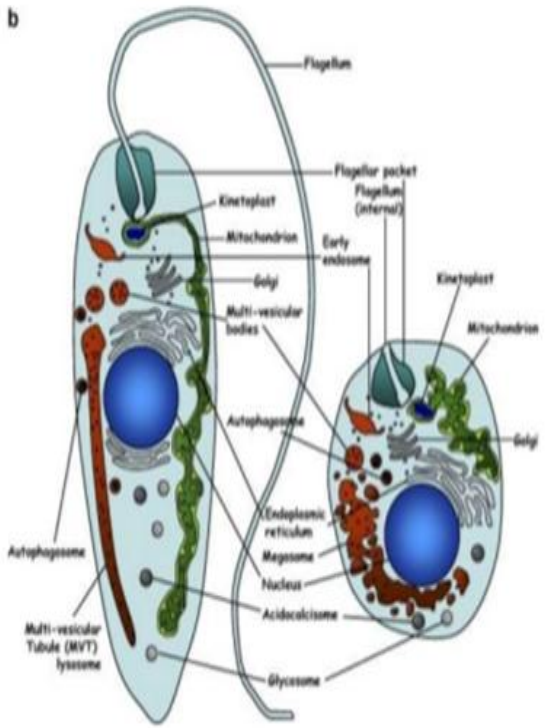
- **Leishmaniasis**:
- is the second-largest parasitic killer in the world (after malaria) and is endemic in many parts of Africa, Asia and South America.

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.

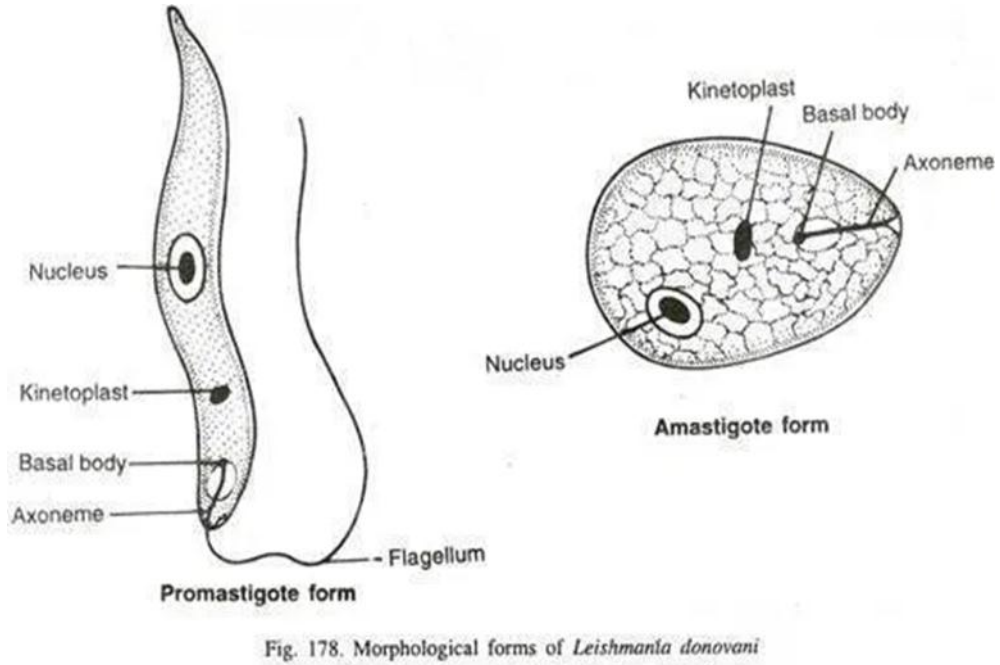


Fig. 178. Morphological forms of *Leishmania donovani*

IMPORTANT SPECIES

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

MODE OF TRAMSMISSION

(*L.donovani*)

Mainly by the bite of sand fly (vector) Phlebotomus argentipus

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

(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.

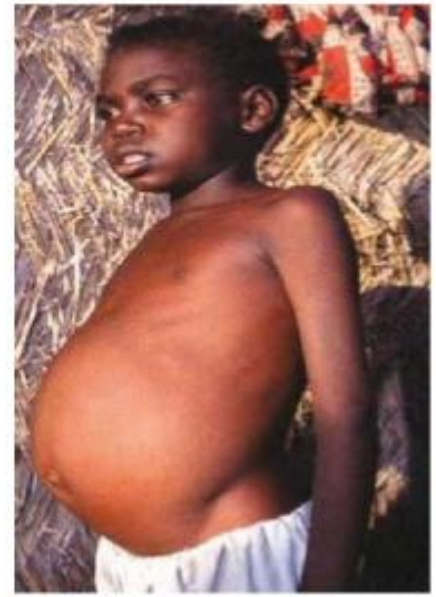
TYPES OF LEISHMANIASIS

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

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*

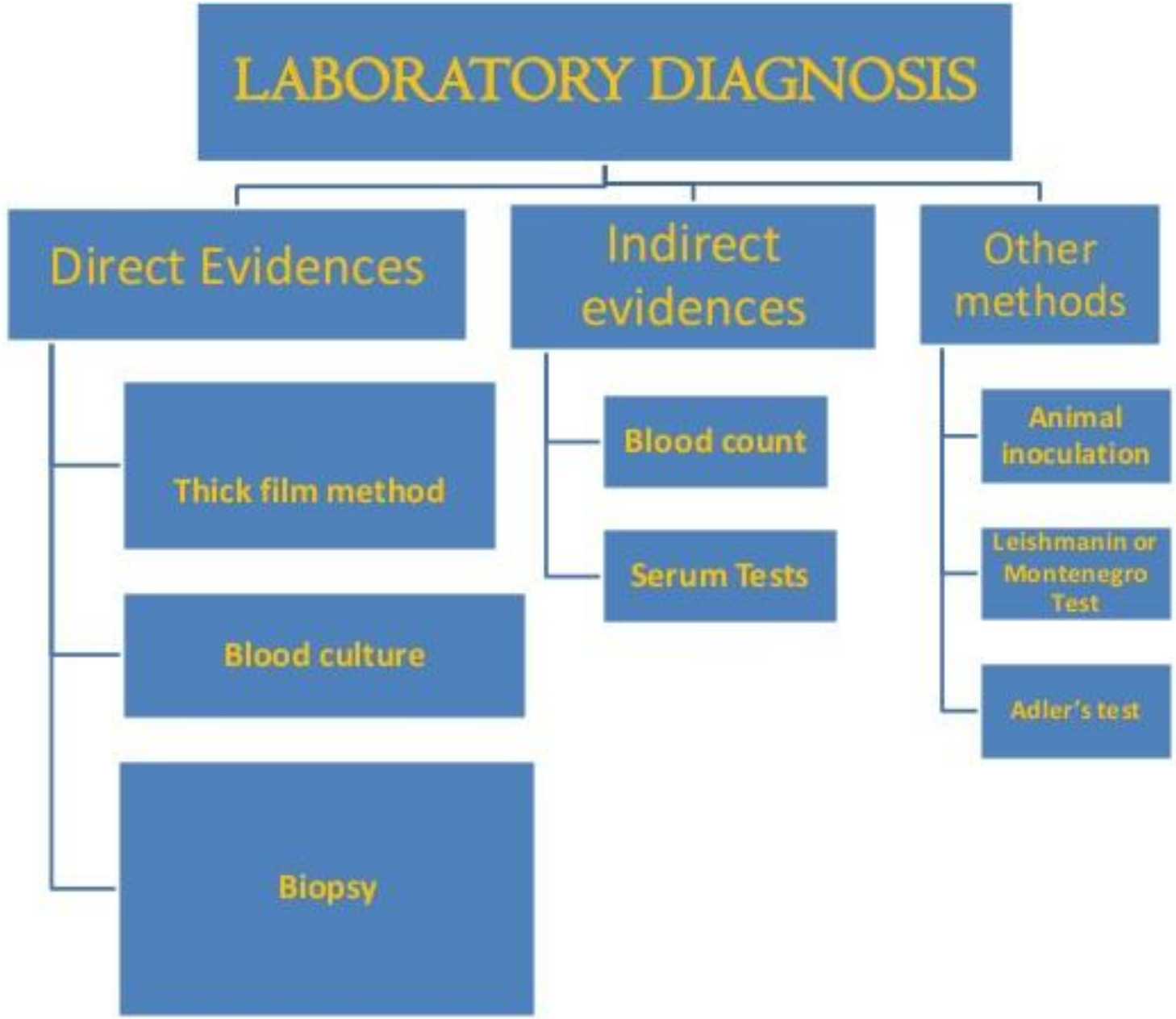
c) **Dermal leishmanoid** or **Post kala-azar dermal leishmaniasis(PKDL):-** caused by *L.donovani*

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

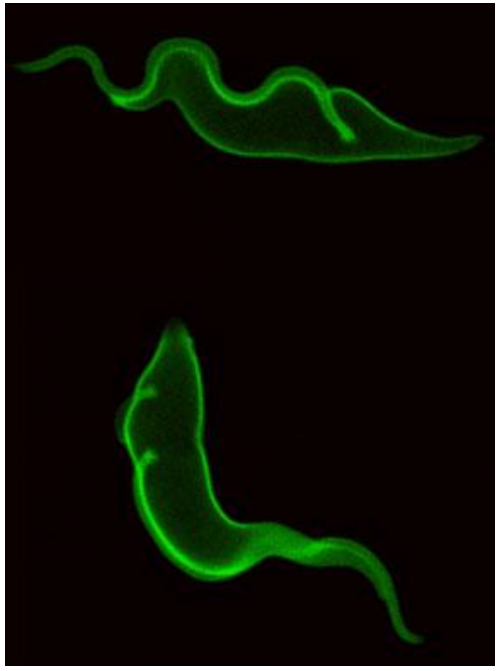
Mucocutaneous leishmaniasis(MCL)

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

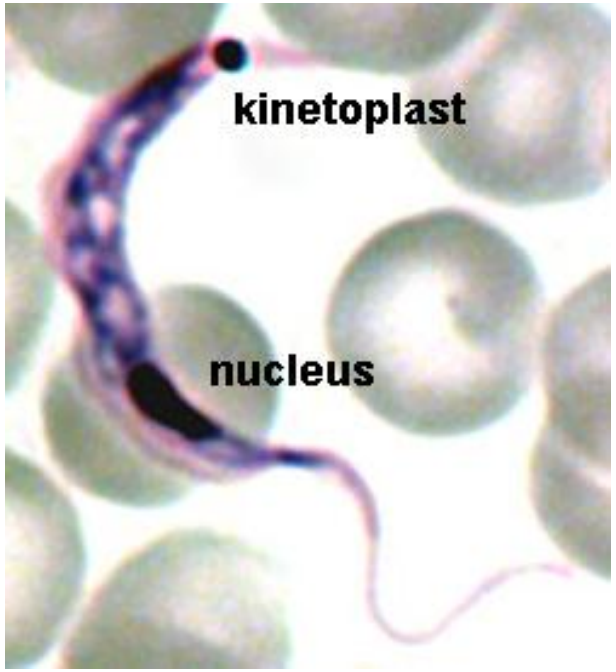
Trypanosomiasis.
Sleeping sickness.



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

Classification:

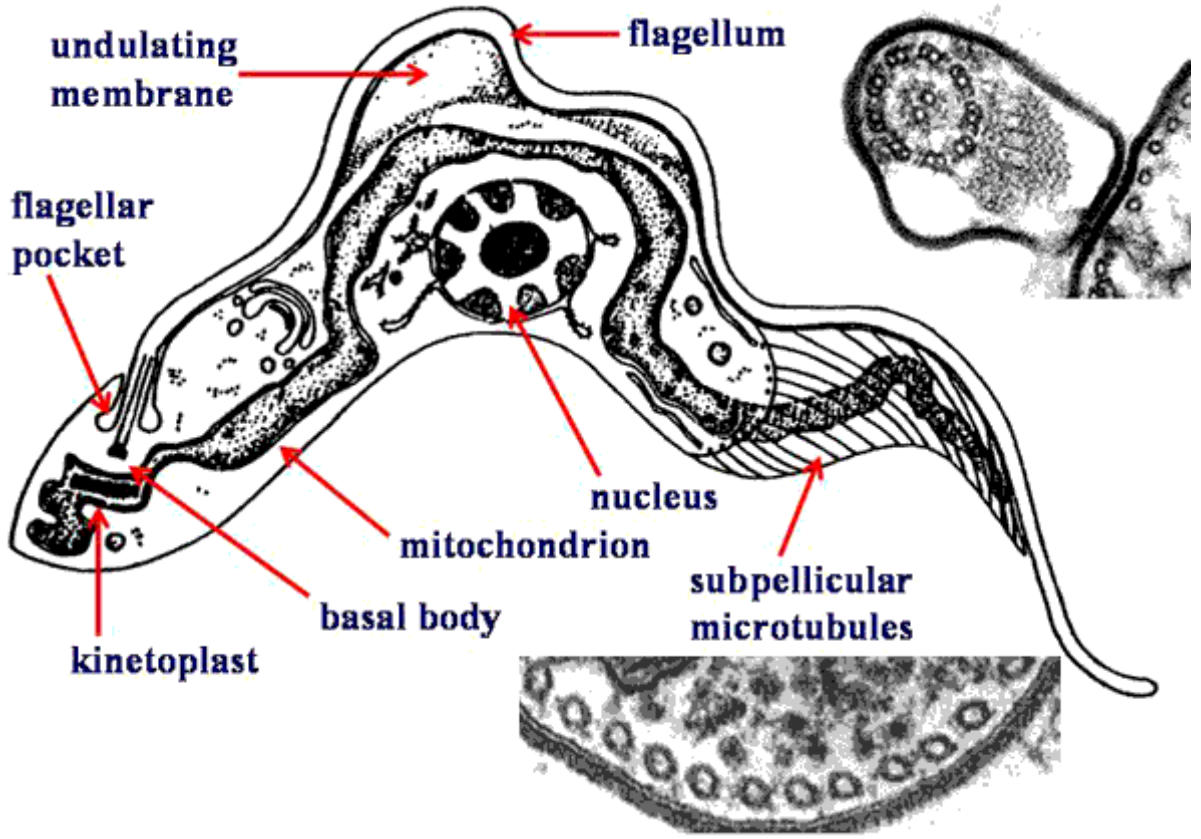
- Eukaryota (organisms with nucleated cells),Kingdom Protista, Phylum Protozoa.
- East African trypanosomiasis is caused by the parasite Trypanosoma brucei rhodesiense.
- West African trypanosomiasis is caused by Trypanosoma brucei gambiense. The parasites are spread by tsetse flies, found only in Africa.



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).

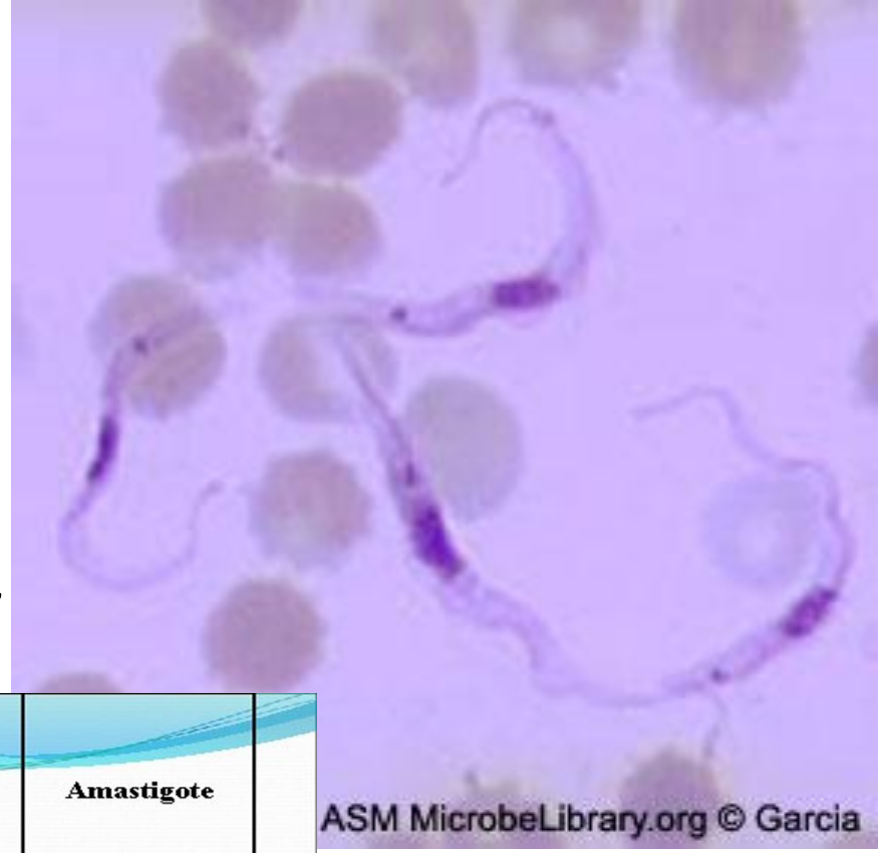
From the point of view of functional and physiologic complexity, a protozoan is more like an animal than like a single cell.

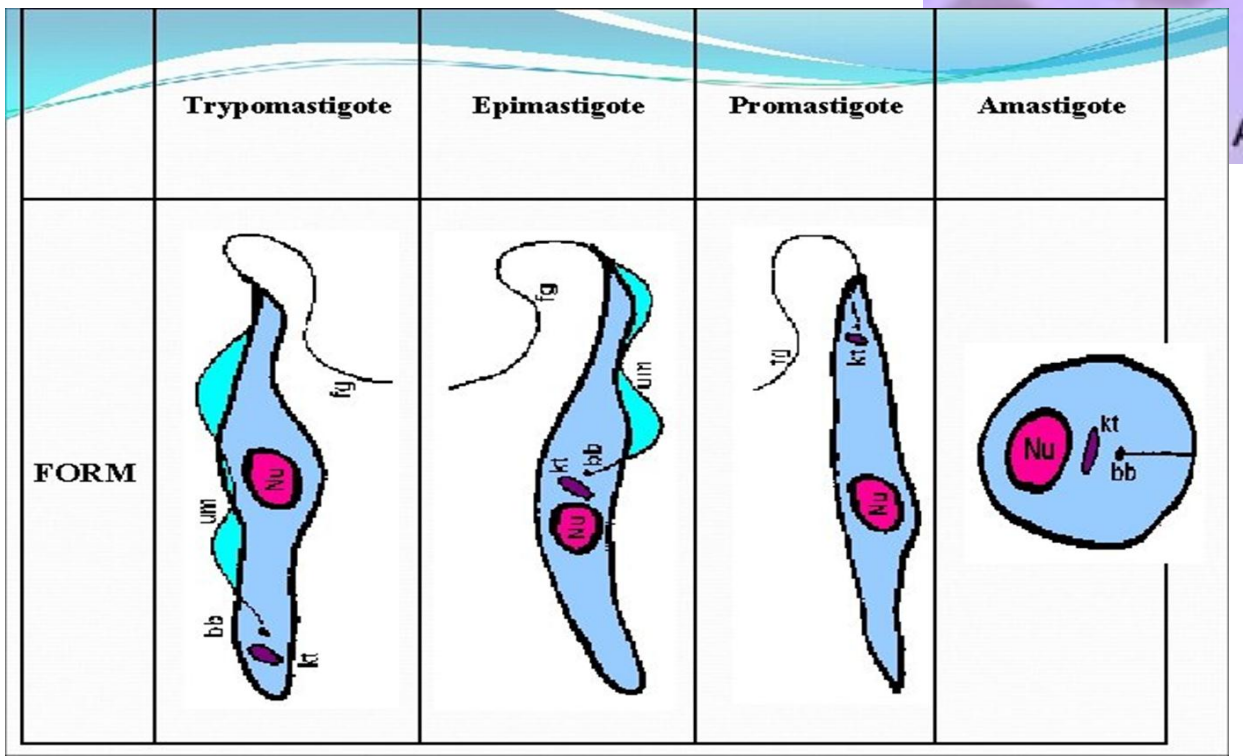


Etiology

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.*

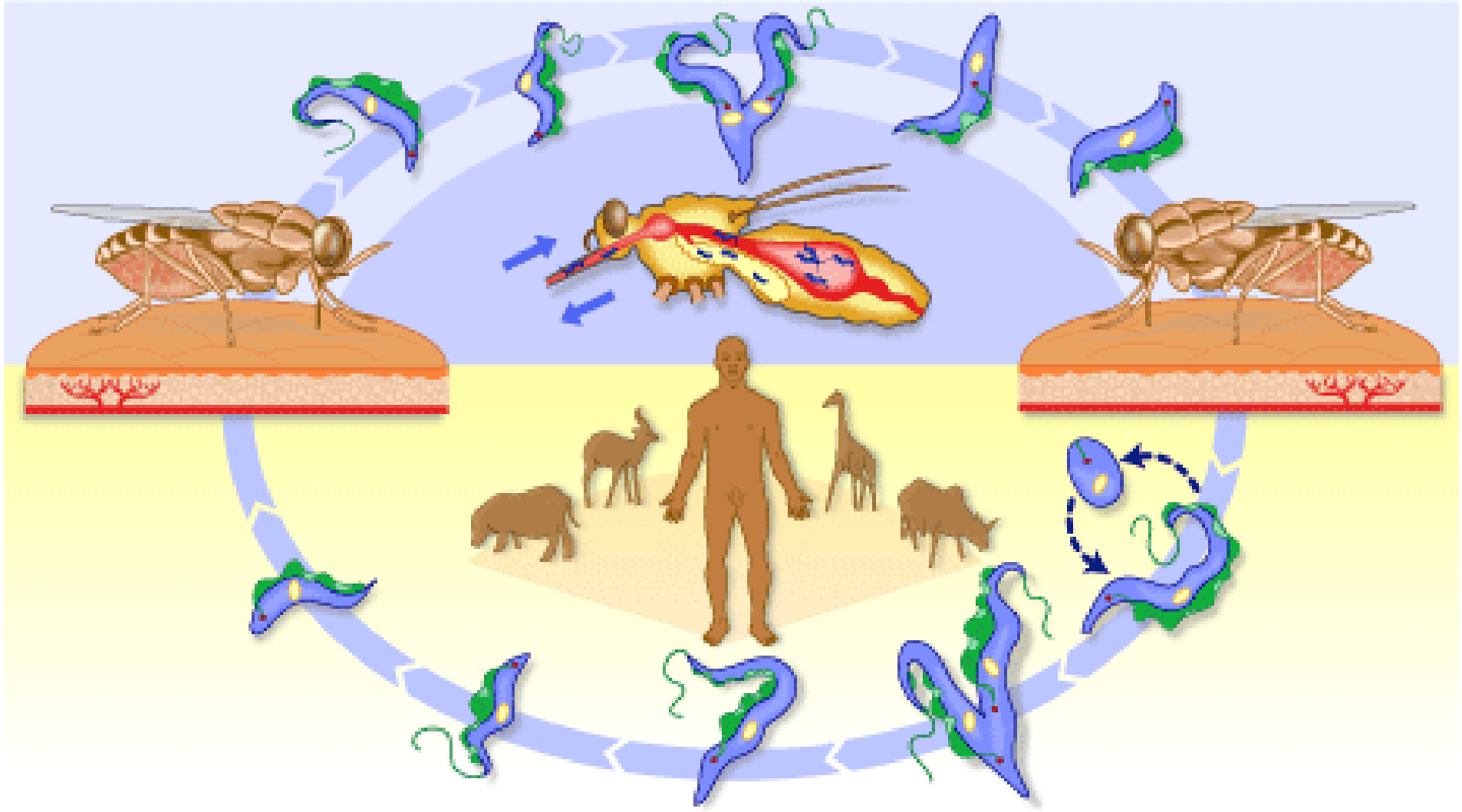




Major Differences Between African Trypanosome Species

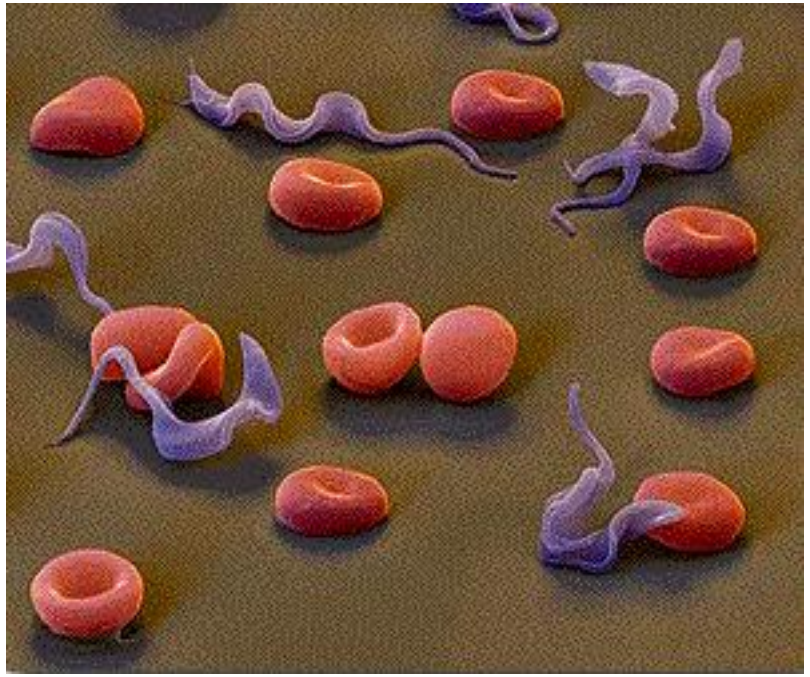
Attribute	***T. rhodesiense***	***T. gambiense***
tsetse vector	*G. morsitans* group	*G. palpalis* group
ecology	dry bush, woodland	rainforest, riverine, lakes
transmission cycle	ungulate-fly-human	human-fly-human
non-human reservoir	wild animals	domestic animals
epidemiology	sporadic, safaris	endemic, 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.

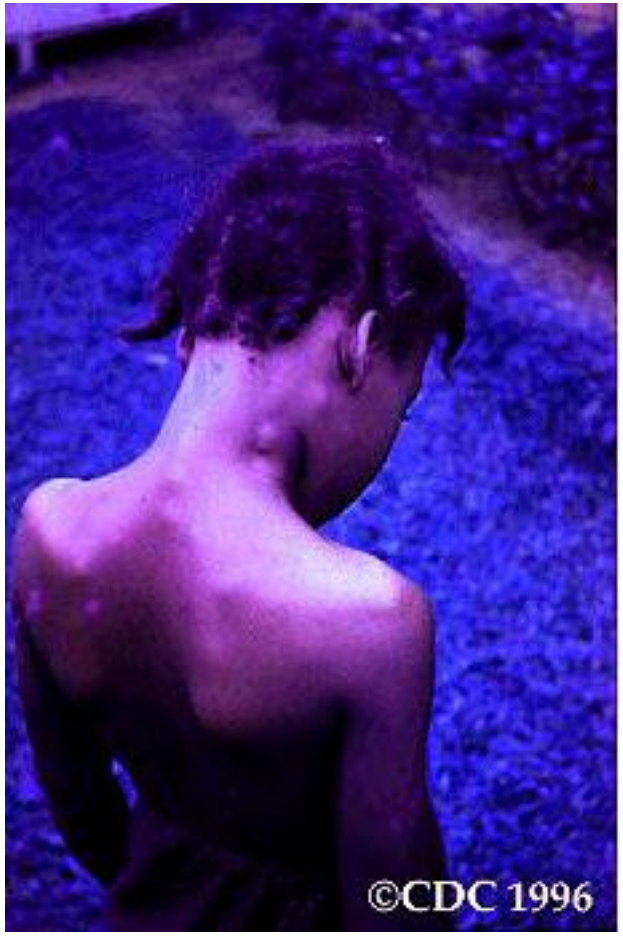


Clinical Symptoms

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

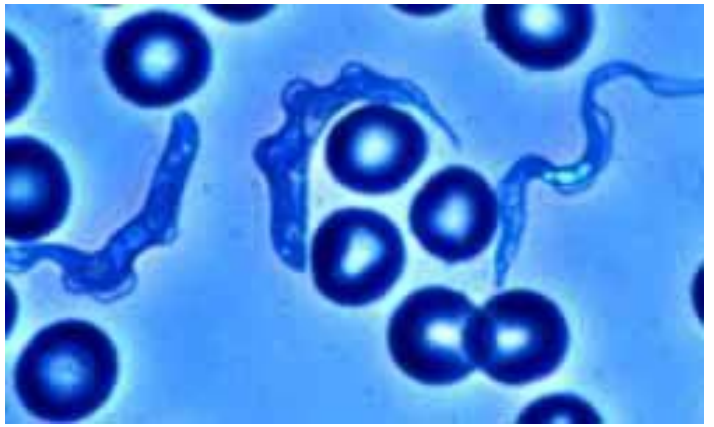
Clinical Symptoms

- 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

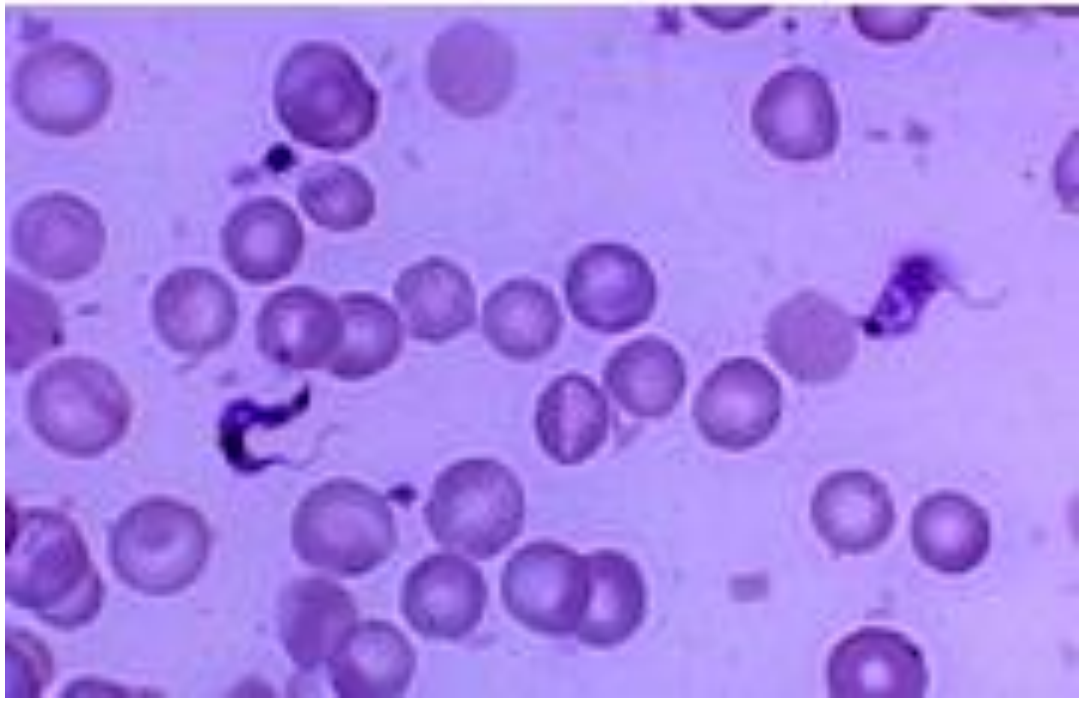
- There is no vaccine or drug to prevent African trypanosomiasis.
- When traveling in areas where the disease occurs, take these precautions against bites from tsetse flies and other insects.

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

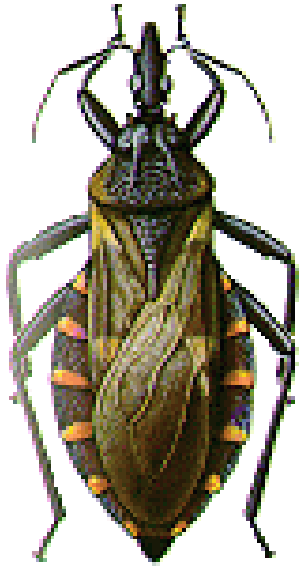
Classification

- 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



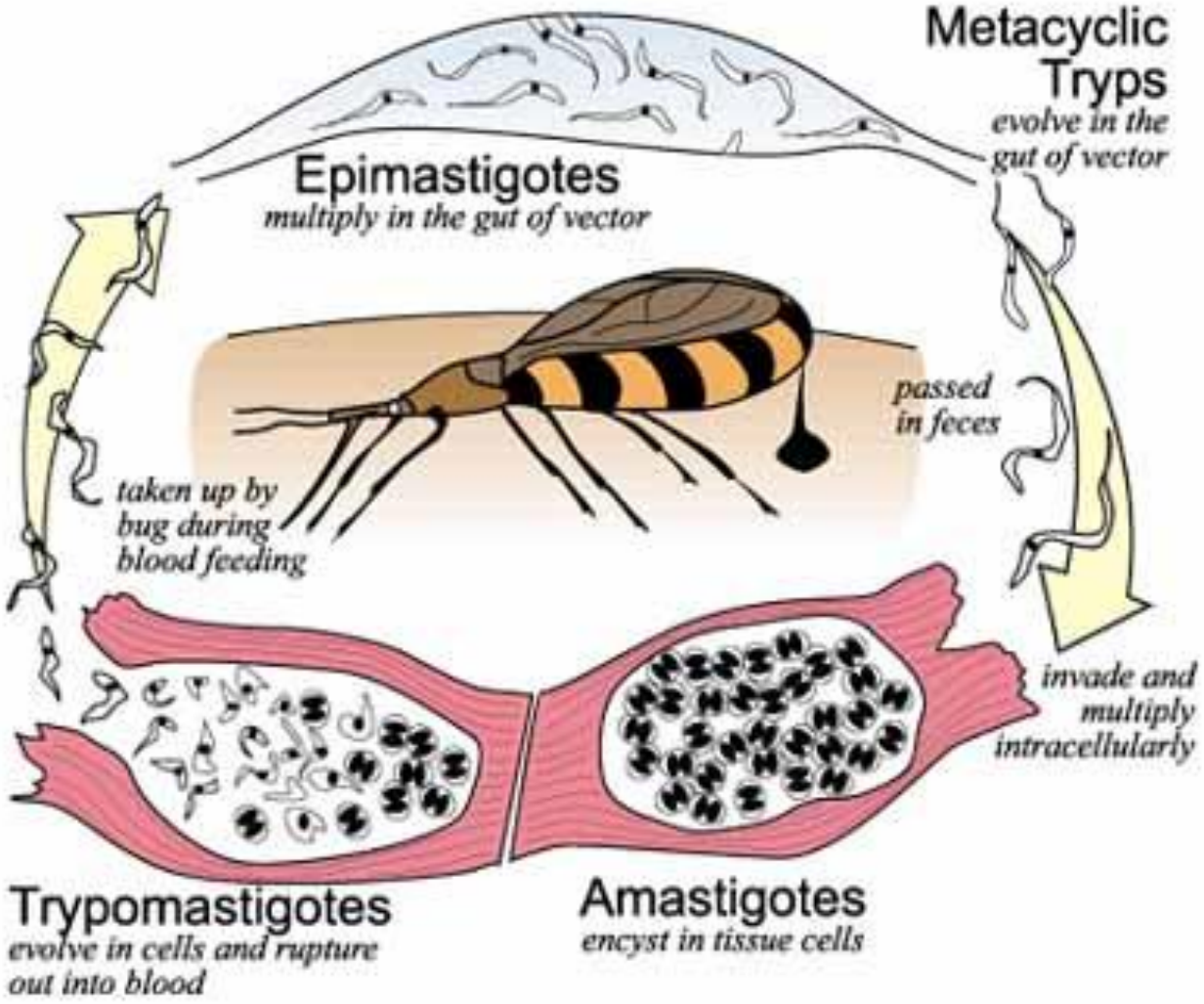
- Chagas disease is transmitted by cone-nosed triatomine bugs of several genera (Triatoma, Rhodnius, Panstrongylus).
- **Trypanosoma cruzi can also be transmitted through blood transfusions, organ transplantation, transplacentally, breast milk and in laboratory accidents.**

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

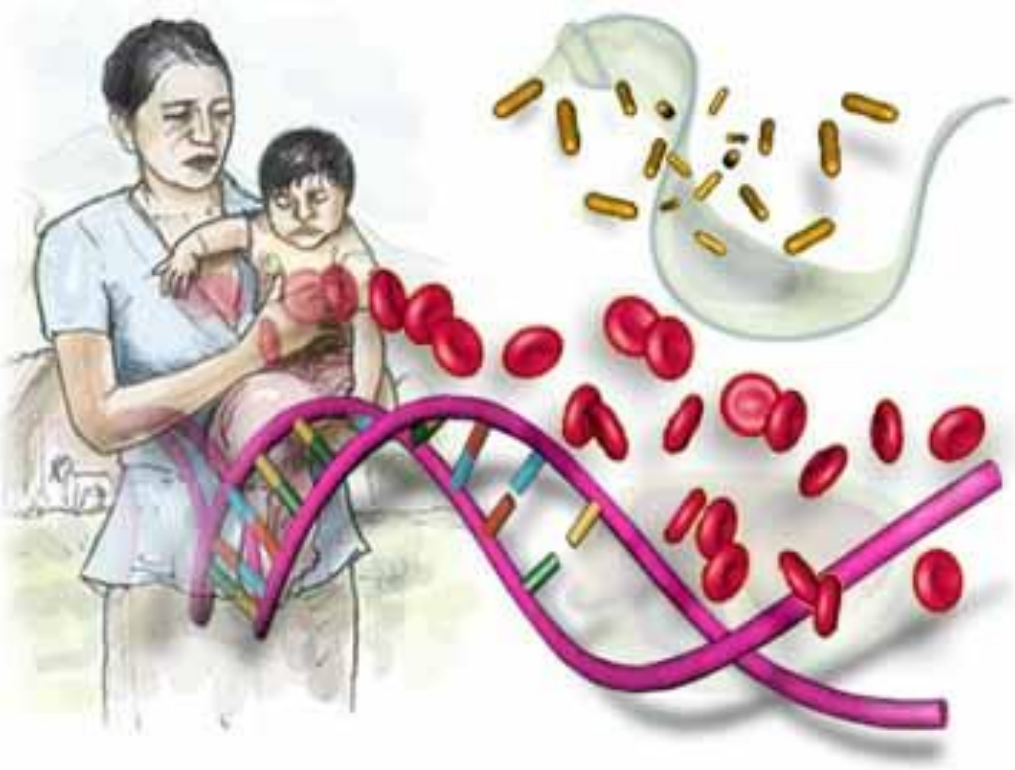
- ***Blood Transfusions and Organ Transplants***

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:

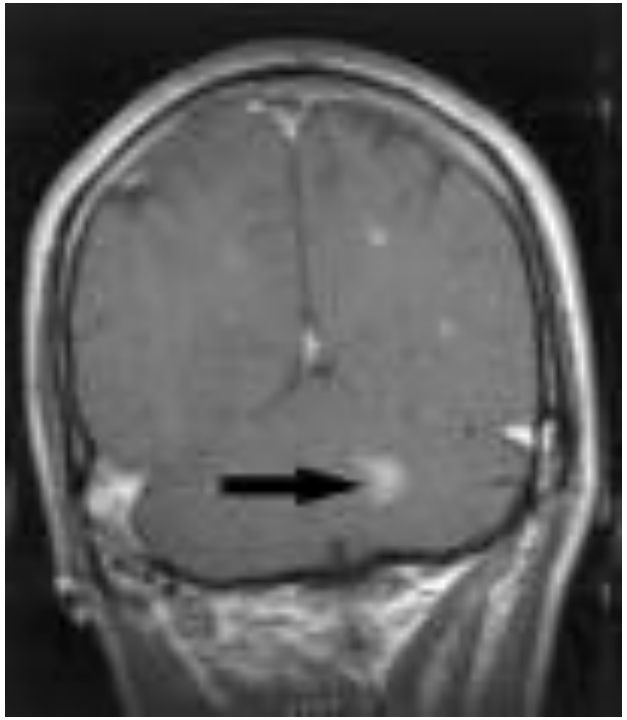
- the acute stage shortly after the infection;
- the indeterminate stage;
- the chronic stage that may develop over 10 years.

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.



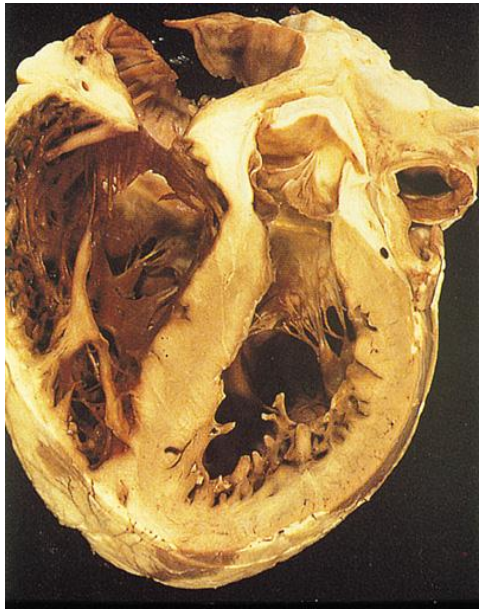
Indeterminate stage

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.



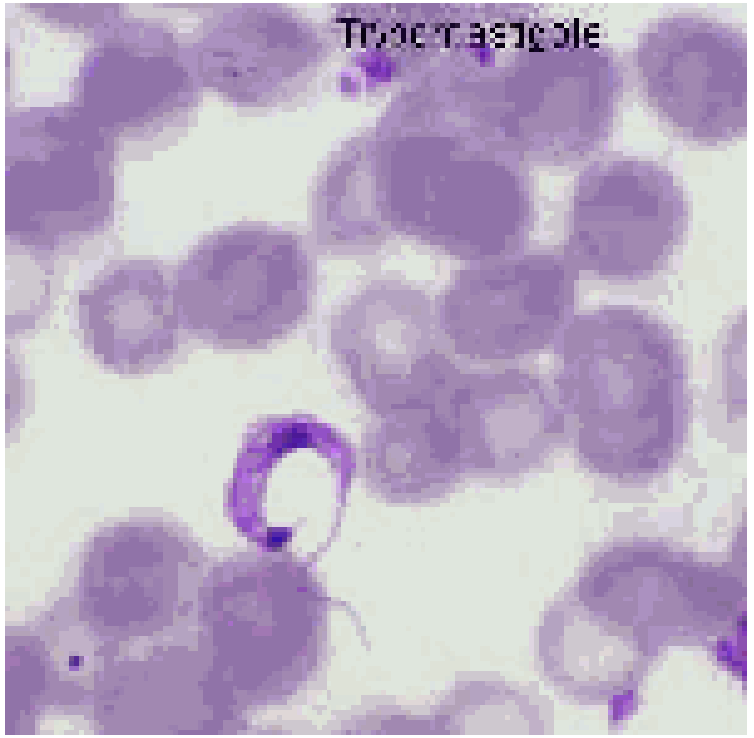
Laboratory Diagnostics

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

➢indirect hemagglutination,

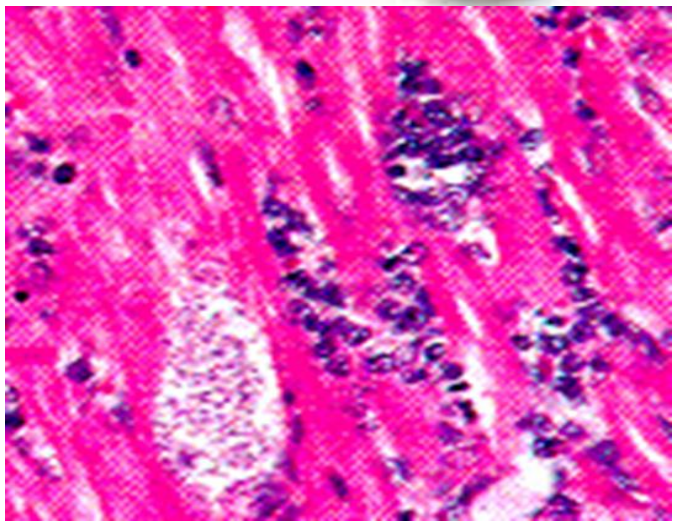
➢indirect immunofluorescence,

➢enzyme-linked immunosorbent assay (ELISA)]

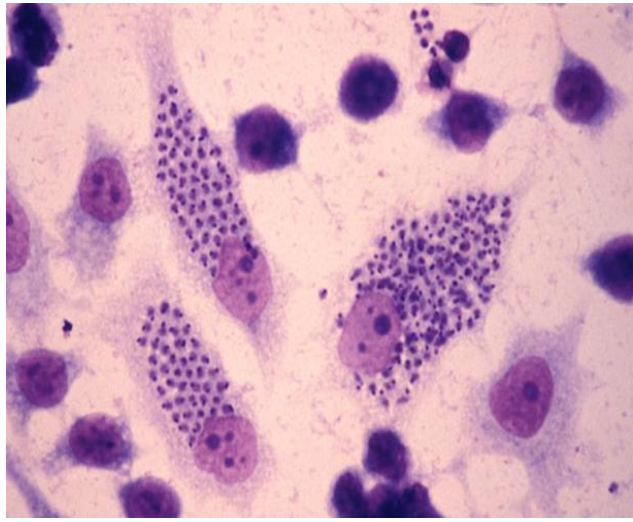


Xenodiagnosis

- 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*.



Amastigotes infecting cells of muscle tissue



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

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.

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