Introduction to Protozoology

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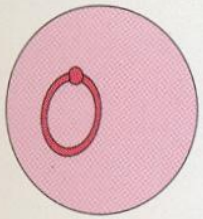
Protozoans are microscopic one-celled organisms that are categorized according to their method of movements.

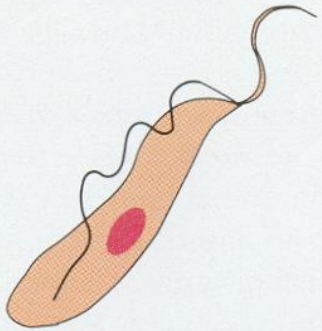
- Ciliates – the only parasitic ciliate that causes disease in humans in *Balantidium coli*
- Flagellates – three of the most common and medically significant include: *Giardia lamblia, Trypanosome* sp. and *Trichomonas vaginalis*
- Amoeba –include the pathogenic amoeba *Entamoeba* and *Endolimax* which cause dysentery in humans
- Apicomplexa – no special organs for movement (*Toxoplasm*a)

THE MAIN DIVISIONS OF PROTOZOA

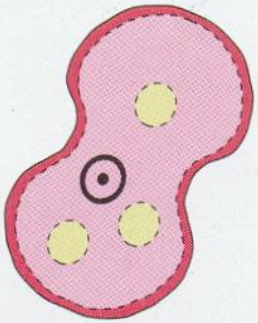
Sporozoa
all are intracellular parasites
e.g. *Plasmodium* in red blood cell



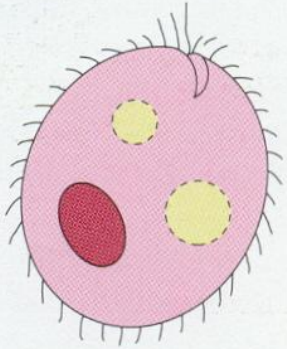
Flagellates
move by beating of one or more flagella
e.g. *Trypanosoma*

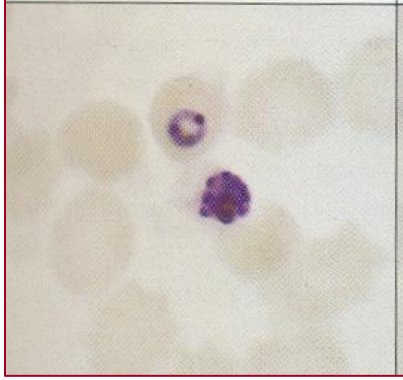


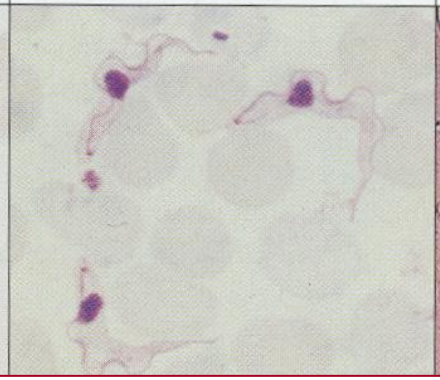
Amoebae
move by extending pseudopodia, no fixed shape, e.g. *Entamoeba*

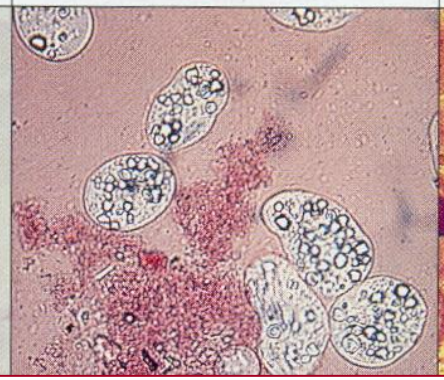


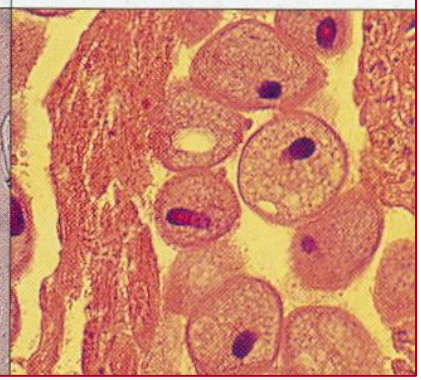
Ciliates
move by beating of many cilia, e.g. *Balantidium*



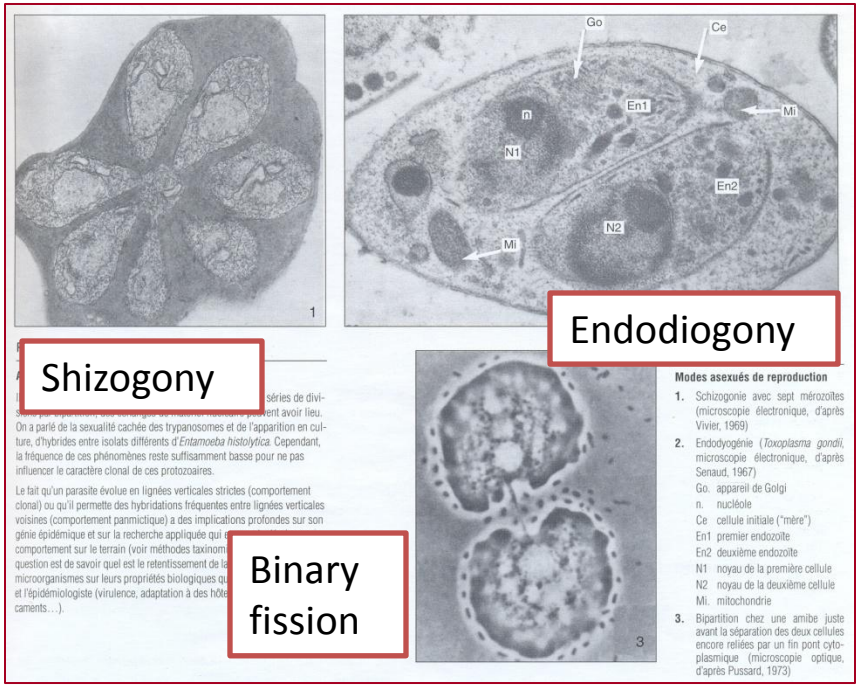








Forms and reproduction

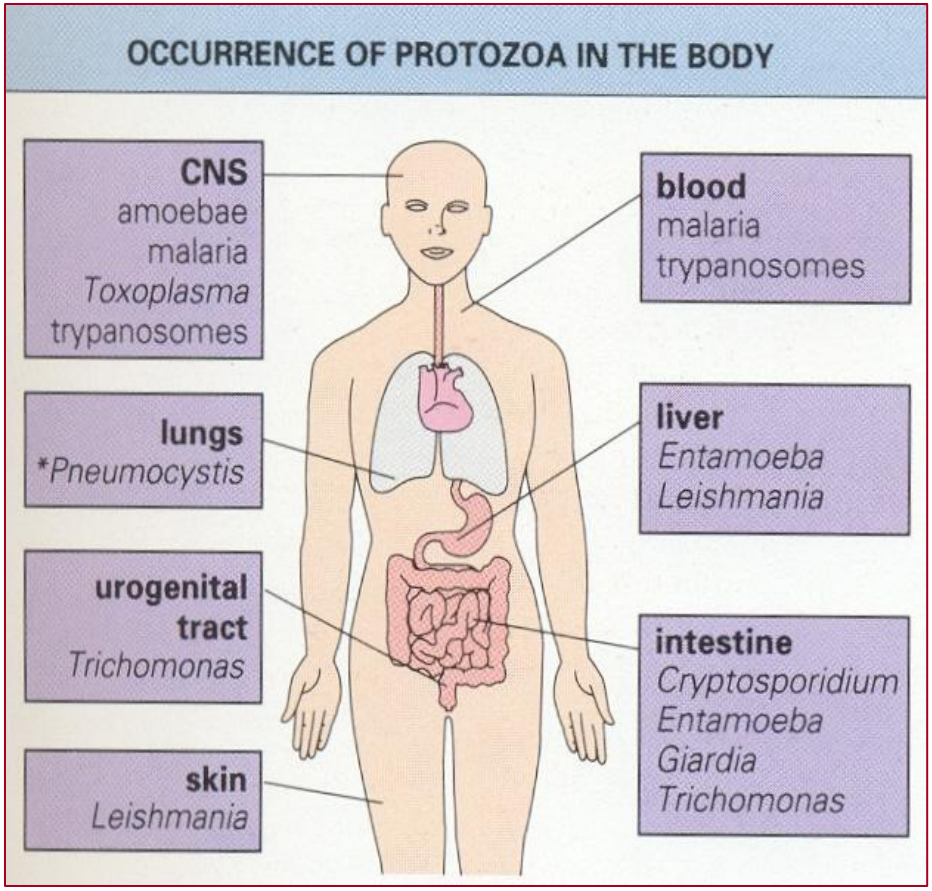


- Cysts - infective forms, survive in the environment
- Trophozoites - vegetative forms, capable for reproduction:
  - ➢ Shizogony (asexual)
  - ➢ Binary fission (asexual)
  - ➢ Endodiogony
  - ➢ Sporogony (sexual)
  - ➢ Conjugation

The Protozoa

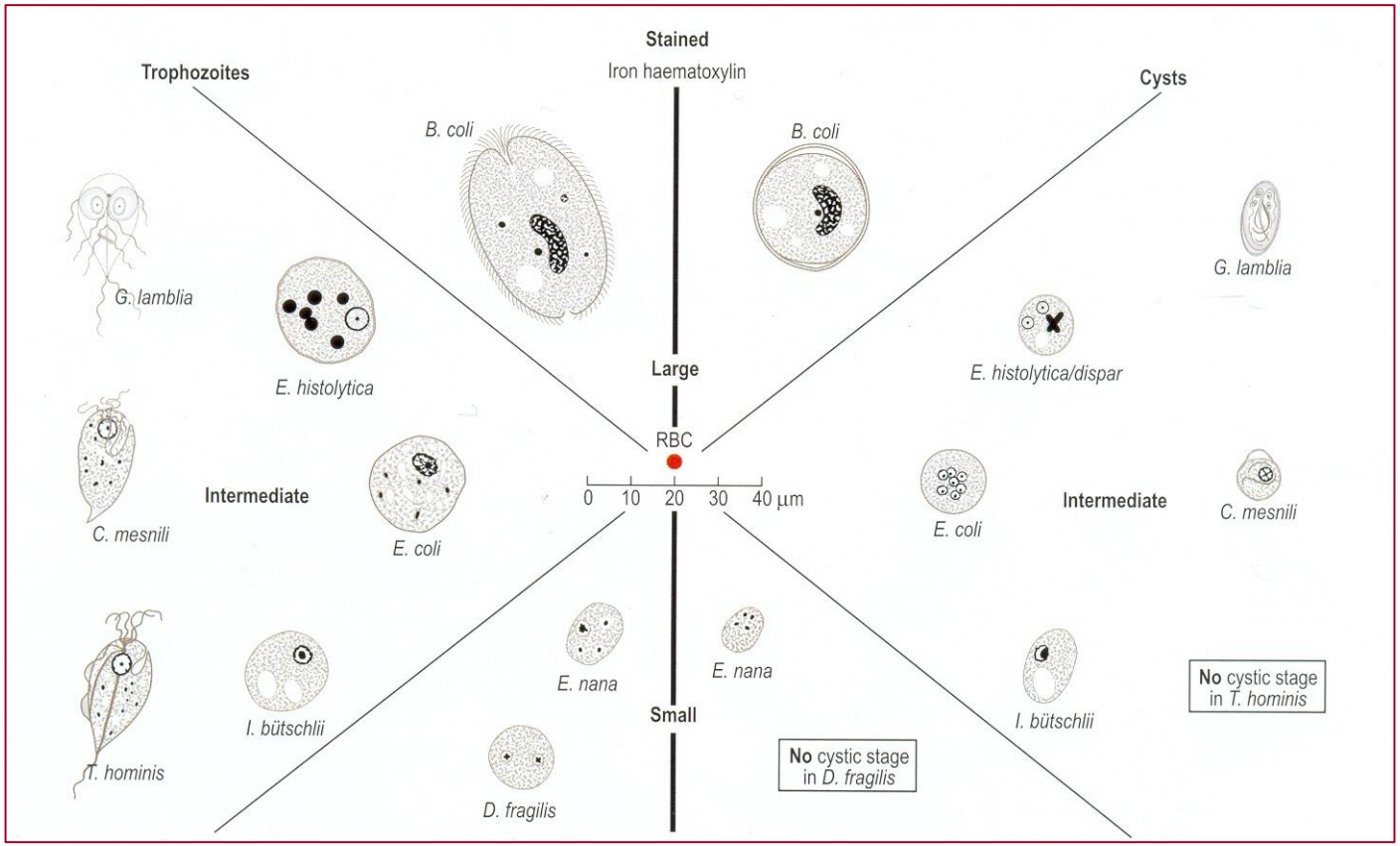
- **Blood and tissue protozoa**
  (e.g.. *Plasmodium* spp.)

- **Intestinal and urogenital protozoa**
  (e.g. *Entamoeba histolytica, Cryptosporidium* spp.)

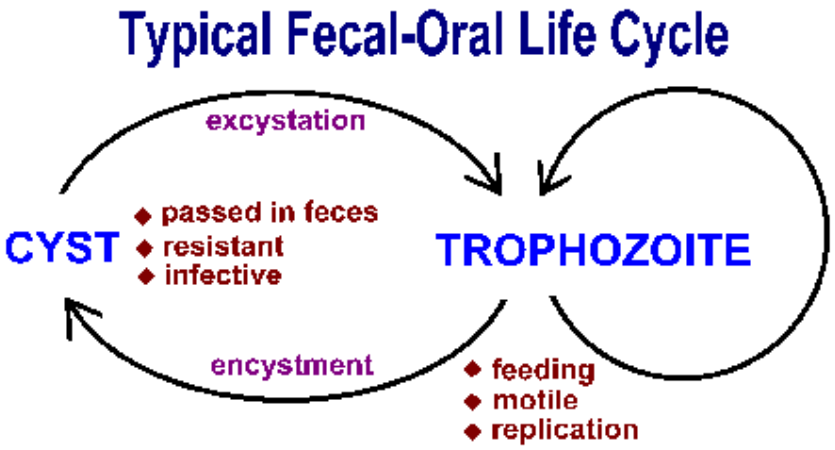


Intestinal and urogenital protozoa

Intestinal protozoa



Intestinal protozoa



Fecal-Oral Transmission Factors

Poor personal hygiene

- children (eg, day-care centers)
- institutions
  (eg, prisons, mental hospitals, orphanages)
- food handlers

Developing countries

- poor sanitation
- lack of indoor plumbing
- endemic
- travelers' diarrhea

Water-borne epidemics

- water treatment failures

Male homosexuality

- oral-anal contact

Zoonosis?

- *Entamoeba* = no
- *Cryptosporidium* = yes
- *Giardia* = controversial

Principle of stool sampling collection, handling and processing for parasites examination

Collection and handling:

- Minimum 3 samples
- Clean, water-tight container with a screw-cap lid
- The smallest acceptable amount of stool is 2-5g
- Urine should not be allowed to contaminate the specimen
- The specimen container should be labeled correctly (patients' name, date and time of sample collection, test/tests requested, suspected diagnosis, clinical findings, travel history)

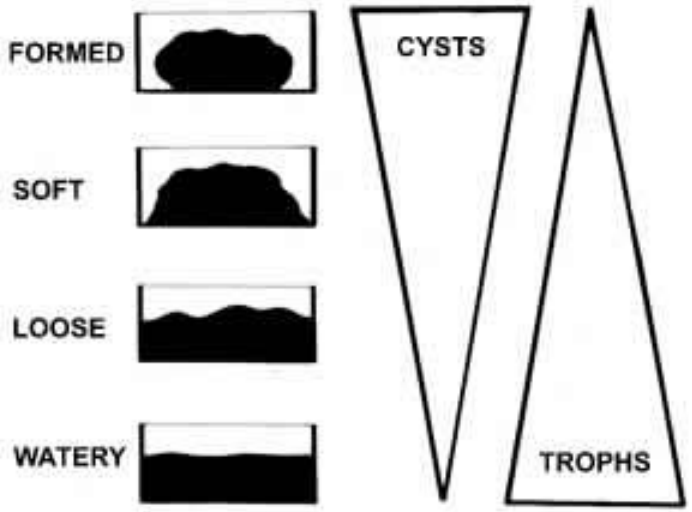
Preservation (fixation)

- The ideal specimen is a freshly collected stool sample
- 5-10% formalin
- PVA – polyvinyl alcohol
- MIF – merthiolate iodine formalin

Processing

- Macroscopic examination:
- consistency
- color
- gross abnormalities
- blood and mucus in feces

- Microscopic examination: standard procedures



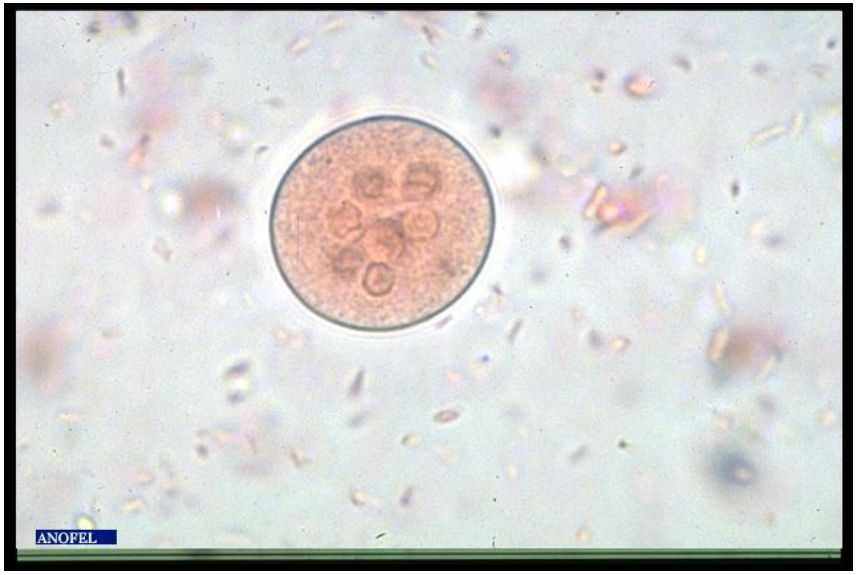
Direct wet preparations

Saline wet preparations:

good for the recovery of the motile protozoan trophozoites

Iodine wet preparations:

study of the detailed morphology of protozoan cysts



Concentration methods

Reason for their use:

(a) removal of debris from the sample

(b) parasites are often present in low numbers and need to be condensed into one area of the sample

- Formalin-ether (or ethyl acetate) concentration procedure: after centrifugation of the sample the parasites present are heavier than solution and settle in the sediment of the tube
- Zinc sulfate flotation technique: after 15min parasites come out on the surface of the solution

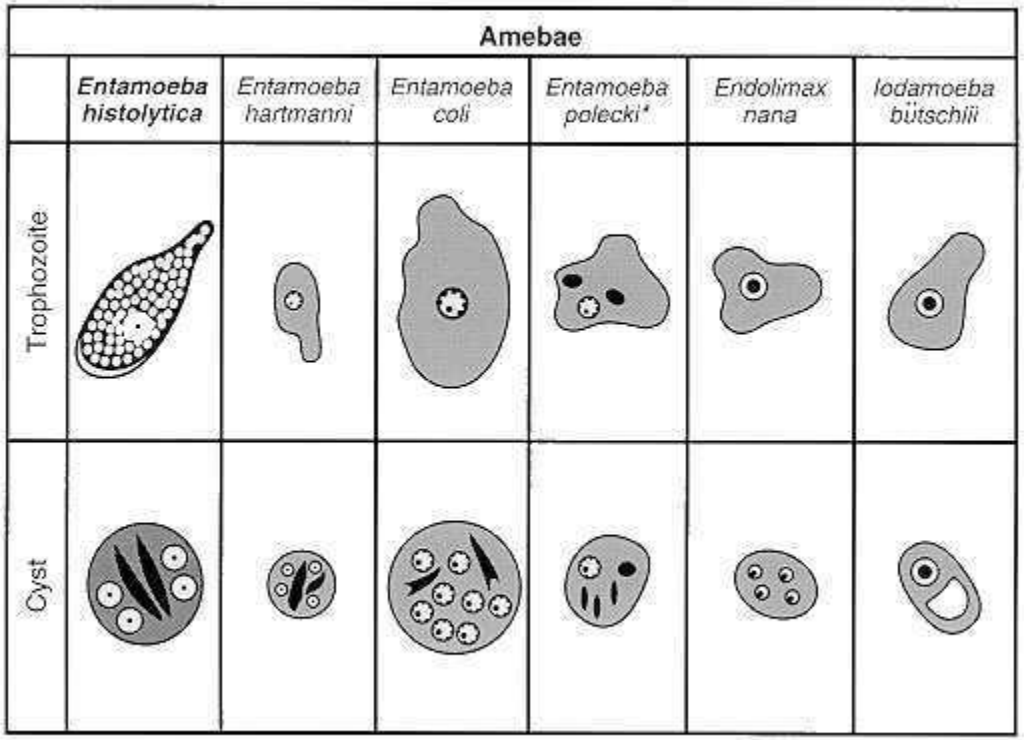
Permanent stains

- Trichrome stain
- Giemsa stain
- Iron hematoxylin stain
- Modified acid-fast stain (modified Ziehl-Neelsen stain)

Immunologic diagnosis

- Detection of Ag from specific parasites in the stool
  (IF, ELISA)

Amebas of human beings



Amebas of human beings

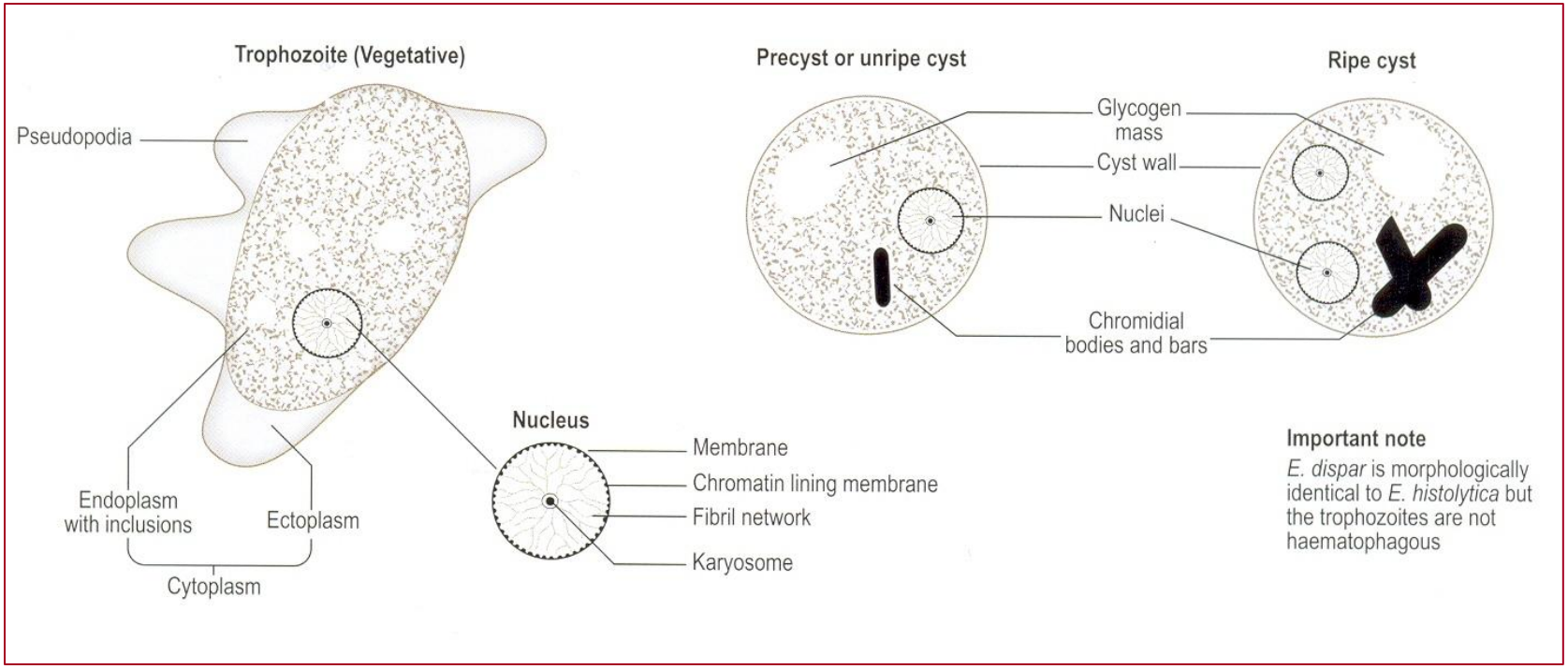
TABLE 79-2 Intestional Amebas of Humans

	Size (μm)			Trophozoite		Cyst	
Organism	Trophozoite	Cyst	Motility (Fresh)	Nuclei (Stained)	(Numbers)	Nuclei Chromatoidals	Remarks
Entamoeba histolytica	10-60	10-20 Round	Active	Karyosome small and central; chromatin, fine and peripheral	1-6	Ends rounded or square	Pathogenic
Entamoeba hartmanni	4-12	5-10 Round	Active	Karyosome small central; chromatin fine and peripheral	1-4	Ends rounded or square	Nonpathogenic
Entamoeba gingivalis	5-35	–	–	Karyosome small central; chromatin fine and peripheral	–	–	Mouth-dwelling nonpathogenic
Entamoeba polecki	10-20	5-10 Round	Sluggish	Karyosome small and central; chromatin variable	1	Ends pointed	Rare in humans nonpathogenic
Entamoeba moshkovskii	10-60	5-20 Round		Karyosome small and central; chromatin fine and peripheral	1-4	Ends rounded	Nonpathogenic
Entamoeba coli	10-50	10-35	Sluggish	Karysome large and eccentic; chromatin clumpy and peripheral	1-8	Ends jagged	Nonpathogenic
Endolimax nana	6-15	4-14 Oval	Sluggish	Karyosome large and variable; little or no chromatin	1-4	None	Nonpathogenic
Iodamoeba bütschlii	6-25	6-20	Active	Karyosome large and central; chromatin absent	1	None	Nonpathogenic

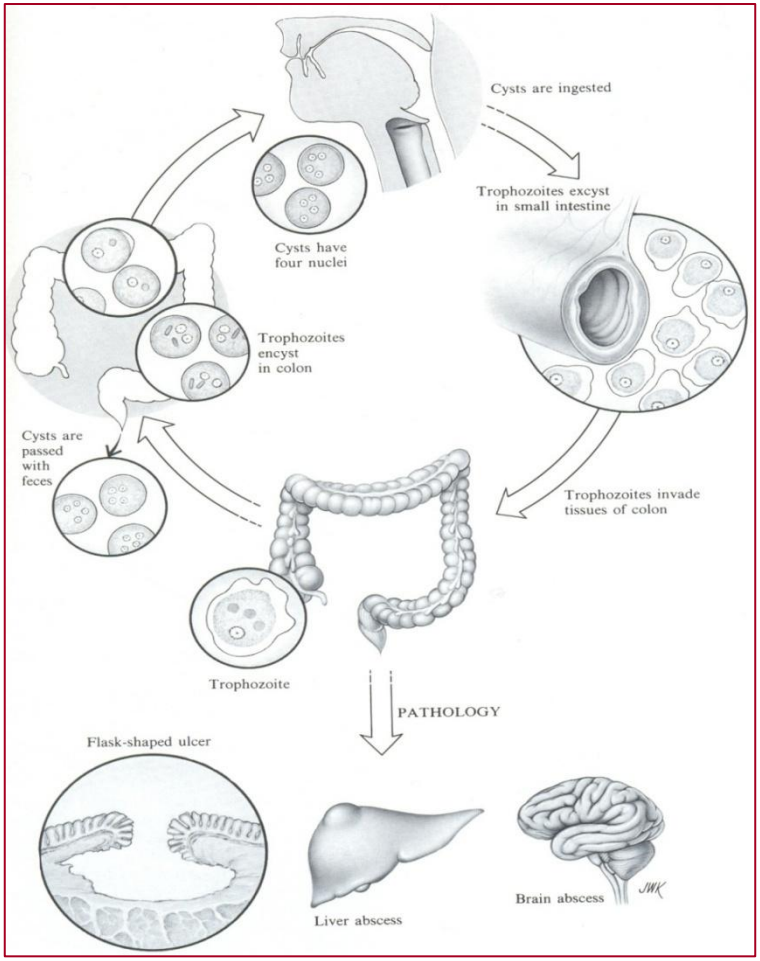
Epidemiology and incidence

- worldwide in distribution, more prevalent in underdeveloped nations with poor sanitation
- the source for infection in humans: contaminated water or vegetables
- cysts are not eradicated with chlorine
- boiling of water is necessary for econtamination
- disease is seen at all ages
- equally distributed in men and women
- invasive disease occurs in 50 million people worldwide each year

Entamoeba histolytica s. dysenteriae



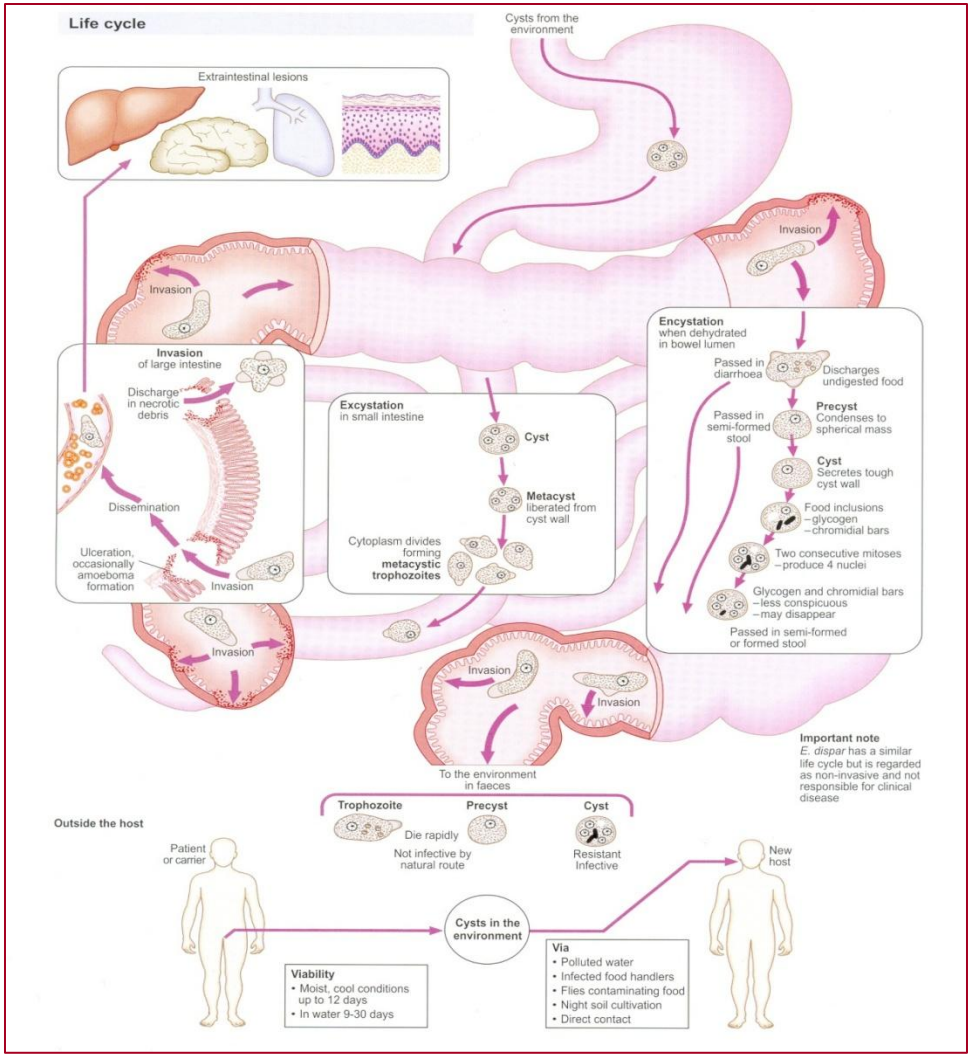
Entamoeba histolytica – life cycle



E. histolytica exhibits a typical fecal-oral life cycle consisting of infectious cysts passed in the feces and trophozoites which replicate within the large intestine.

Trophozoites colonize the large intestine, especially the cecal and sigmoidorectal regions, where they feed on bacteria and cellular debris.

Entamoeba histolytica – life cycle



Possible Virulence Factors

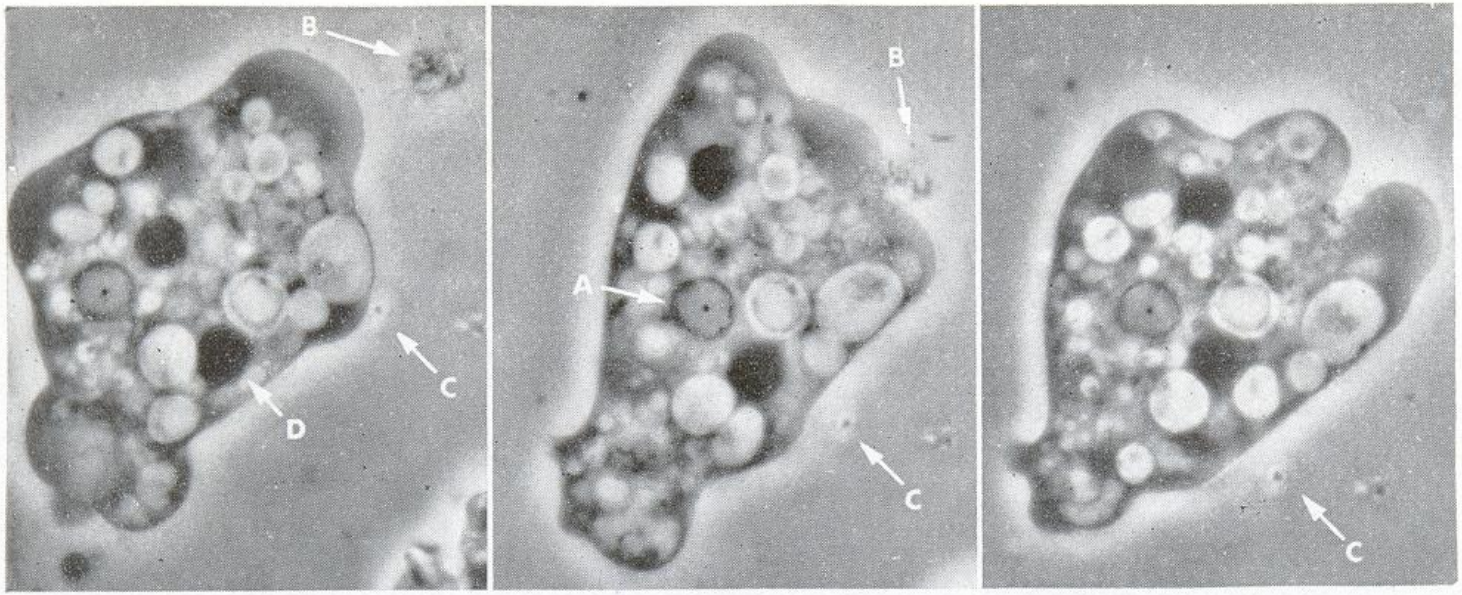
Host factors

- ineffective innate immunity
- inflammatory response

Parasite factors

- resistance to host response
  (eg, complement resistance)
- adherence properties
- cytolytic properties
- ability to breakdown tissues (e.g. secreted proteases)

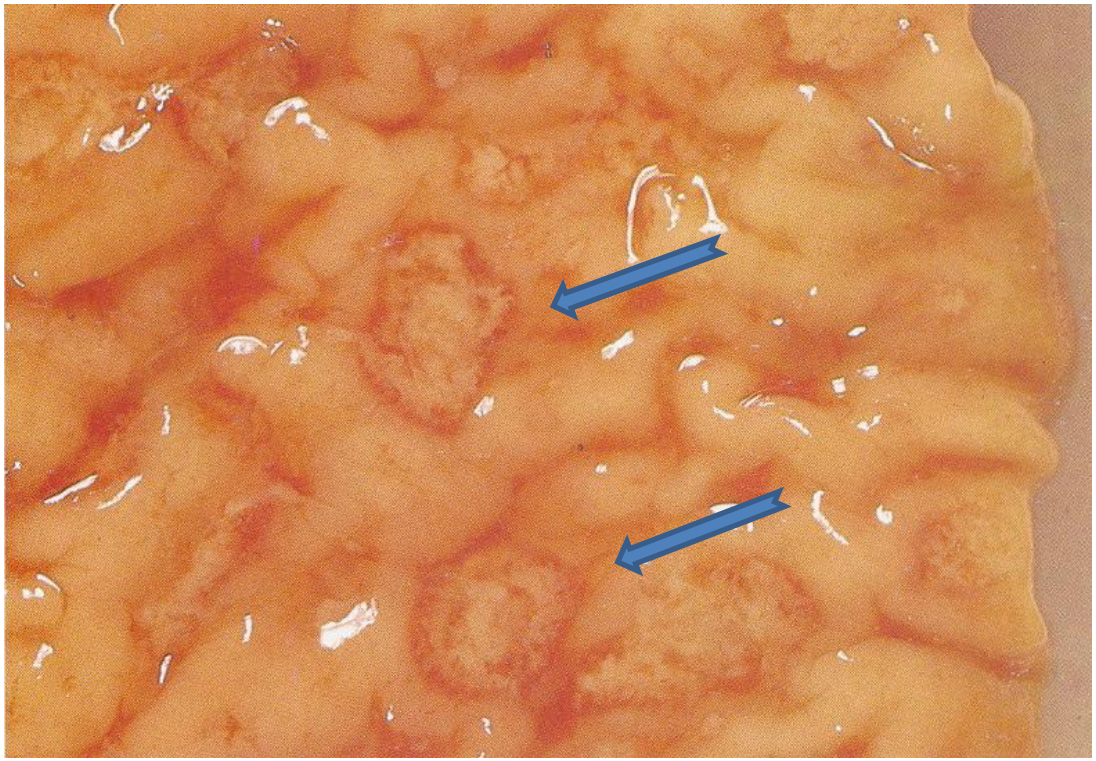
Entamoeba histolytica – trophozoite



Entamoeba histolytica. Time lapse photography under phase contrast. The point at C is a marker against which the movement of the trophozoite can be judged; at point B are particles towards which the trophozoite progresses and eventually engulfs. D indicates a recently ingested red blood cell and at A the characteristic nucleus is evident. ×750

The cells in both of the above series were harvested from a culture in Jones' medium.

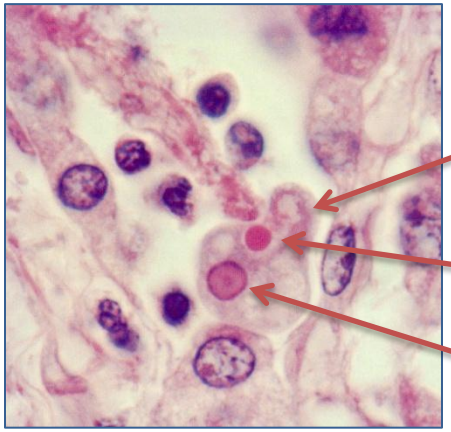
Entamoeba histolytica – colon ulcers

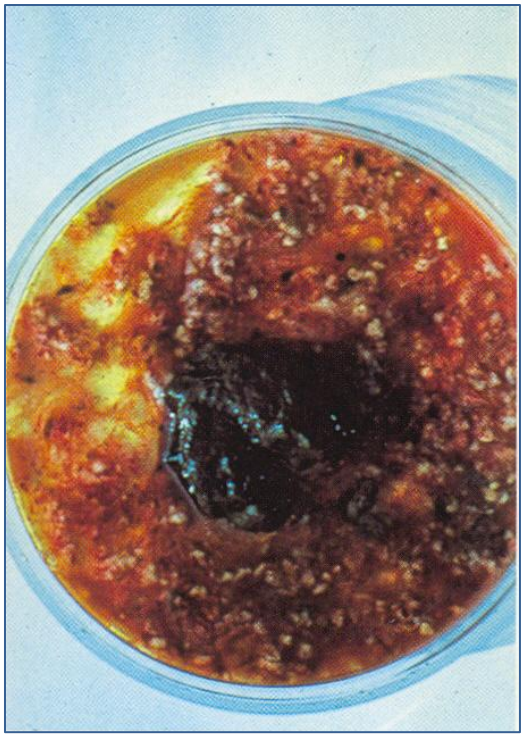


Entamoeba histolytica – intestinal amebiasis

Trophozoites can invade the colonic epithelium and produce ulcers and dysentery

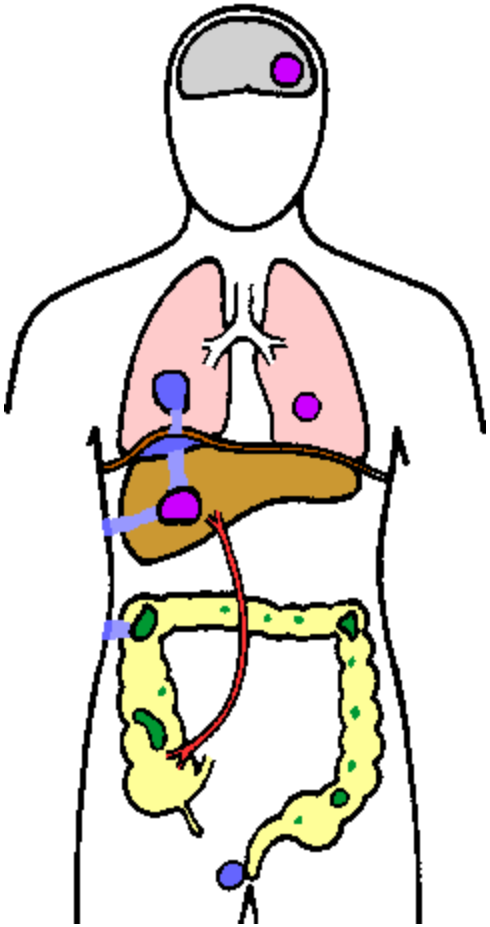
Colon ulcer HP





Blood and mucus in feces (dysentery)

Extraintestinal amebiasis



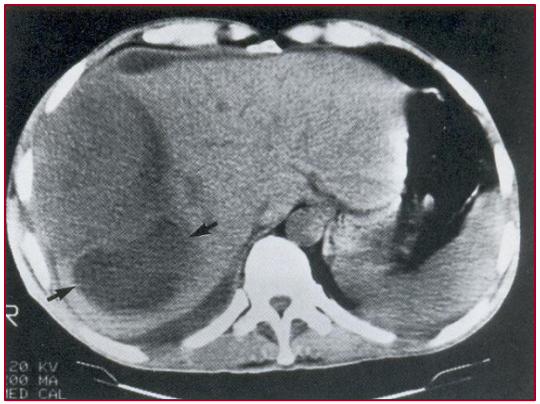
E. histolytica is found primarily in the colon where it can live as a non-pathogenic commensal or invade the intestinal mucosa (green).

The ameba can metastasize to other organs via a hematogenous route (purple), primarily involving the portal vein and liver.

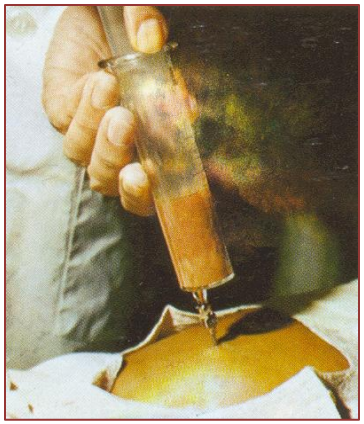
The ameba can also spread via a direct expansion (blue) causing a pulmonary infection, cutaneous lesions or perianal ulcers

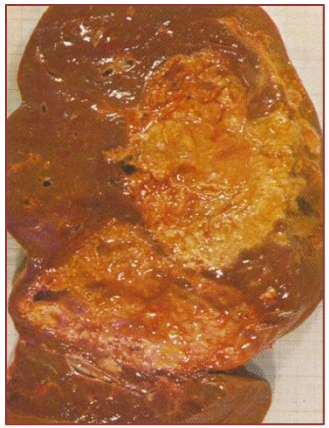
Extraintestinal amebiasis

Abscess in liver

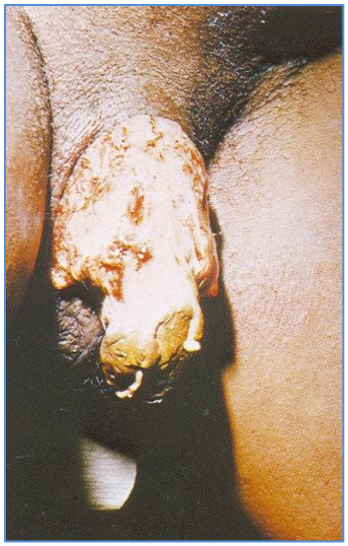


Aspirate from liver abscess





Cutaneous amebiasis



Ano-rectal amebiasis

Genital amebiasis

Incubation period

- patients develop symptoms with invasive disease within 3 weeks of ingestion of the cysts
- amebic liver abscess formation takes about 3 months to develop
- some patients apparently carry the organisms for prolonged periods before developing significant clinical manifestations

Clinical Syndromes Associated with Amebiasis

Intestinal Disease

- asymptomatic cyst passer
- symptomatic nondysenteric infection
- amebic dysentery (acute)
- fulminant colitis + perforation (peritonitis)
- ameboma (amebic granuloma)
- perianal ulceration

Extraintestinal Disease

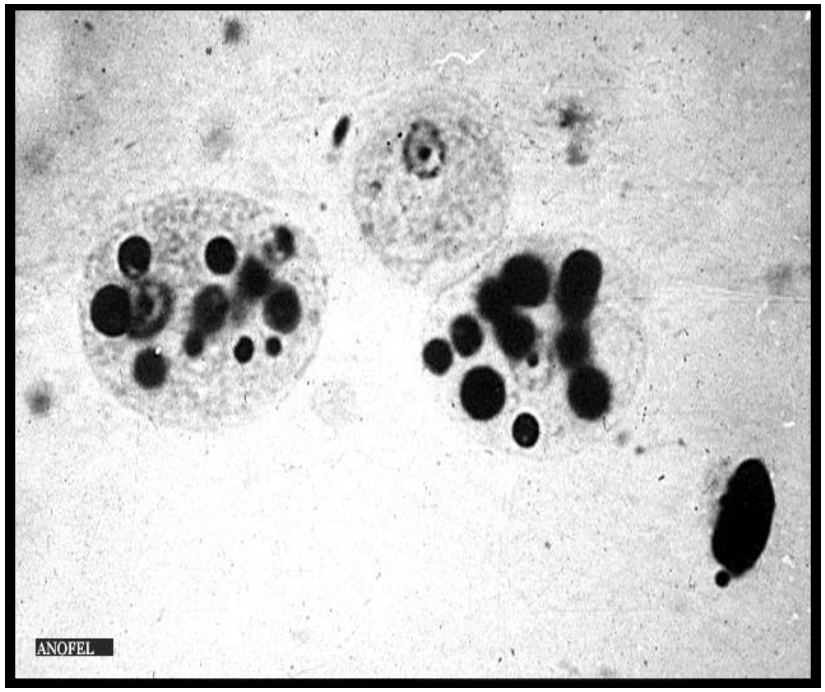
- liver abscess
- pleuropulmonary amebiasis
- brain and other organs
- cutaneous and genital diseases

Diagnosis of intestinal amebiasis

Intestinal Disease

- stool examination
  - cysts and/or trophozoites
- sigmoidoscopy
  - lesions, aspirate, biopsy
- antigen detection
  - histolytica/dispar

E. histolytica - trophozoites



Heidenhein stain

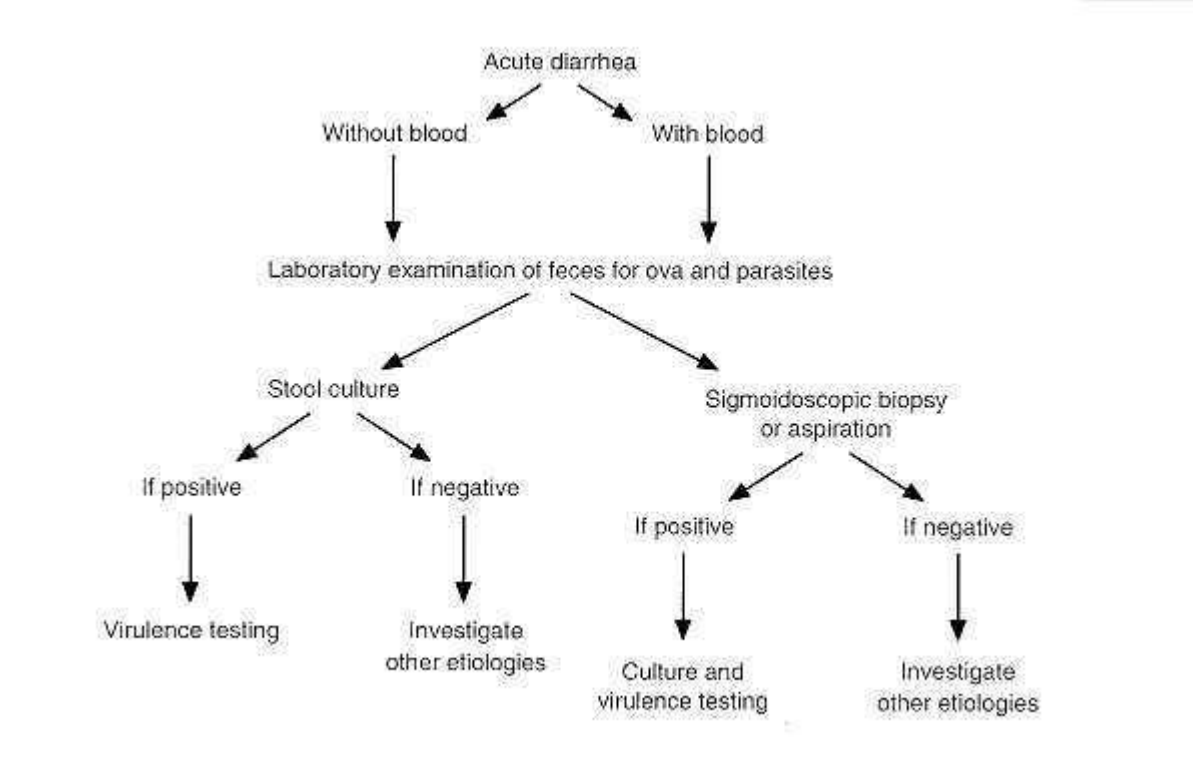
Wet mount

E. histolytica – cyst

E. coli cyst *(*Iodine wet preparation)

E. hisolytica cyst (wet mount)

Diagnosis of intestinal amebiasis



Amebiasis - diagnosis

- all patients with invasive disease have blood in the stools
- cysts or trophozoites should be visible on microscopic evaluation of the stool
- colonic biopsy specimens reveal organisms
- antiamebic antibodies are positive in patients with invasive disease only
- leukocytosis without eosinophilia is often seen in patients with invasive amebic disease
- elevated liver function tests can be seen in cases of liver involvement

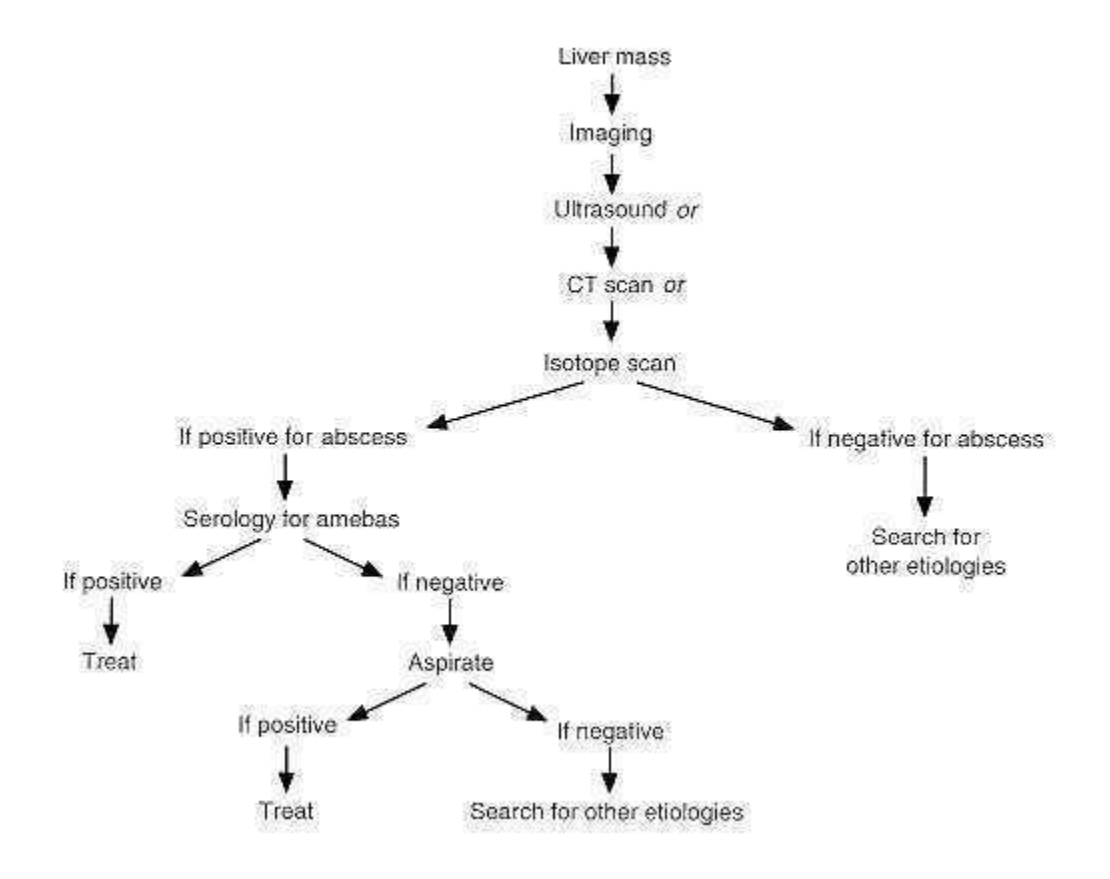
Diagnosis of extraintestinal amebiasis

Extraintestinal (hepatic) Disease

- serology
  - current or past?
- imaging
  - CT, MRI, ultrasound
- abscess aspiration
  - only select cases
  - reddish brown liquid
  - trophozoites at abscess wall

  ➢ aspiration of a liver abscess often fails to recover the organism, since it lives in the walls of the abscess

Diagnosis of extraintestinal amebiasis



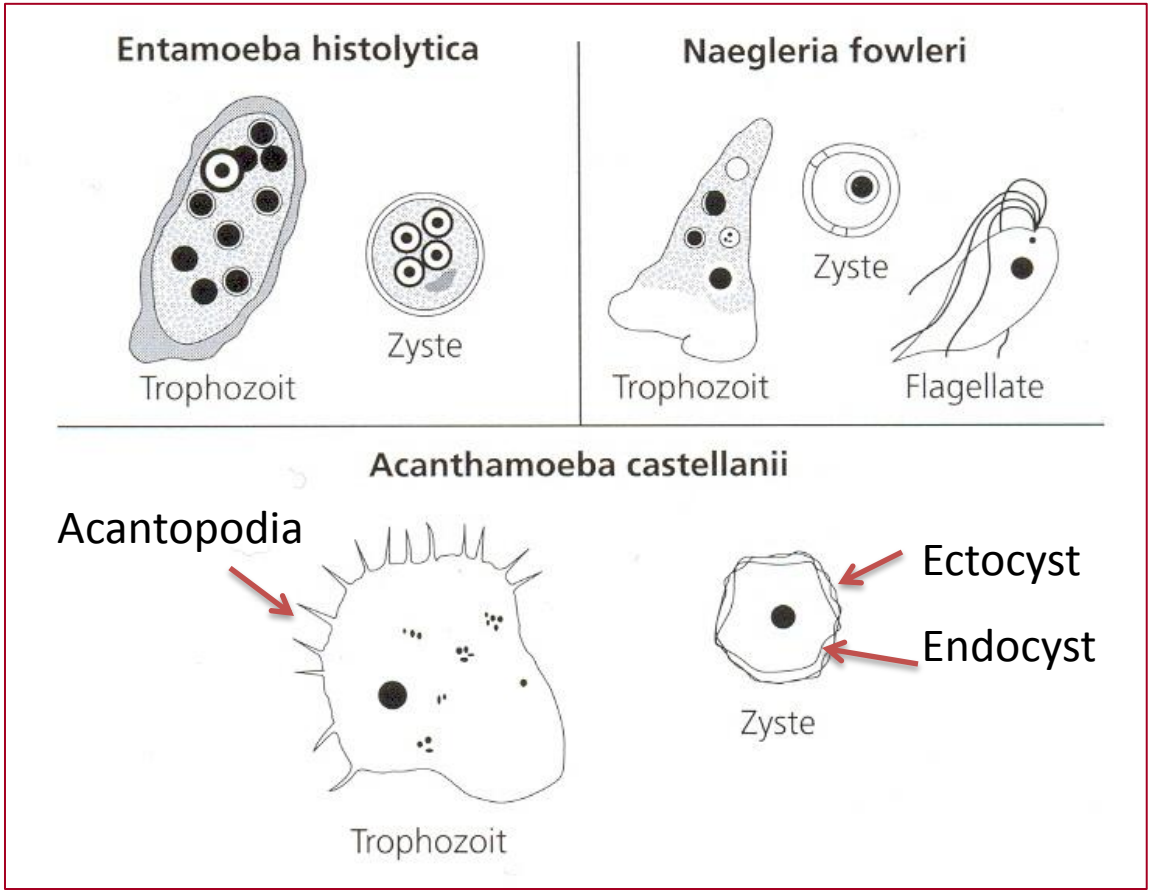
Entamoeba coli

- nonpathogenic comensal
- trophozoites do not ingest erytrocytes and do not invade tissues
- the cyst has 8 nuclei (versus 4 nuclei of *E. histolytica*)

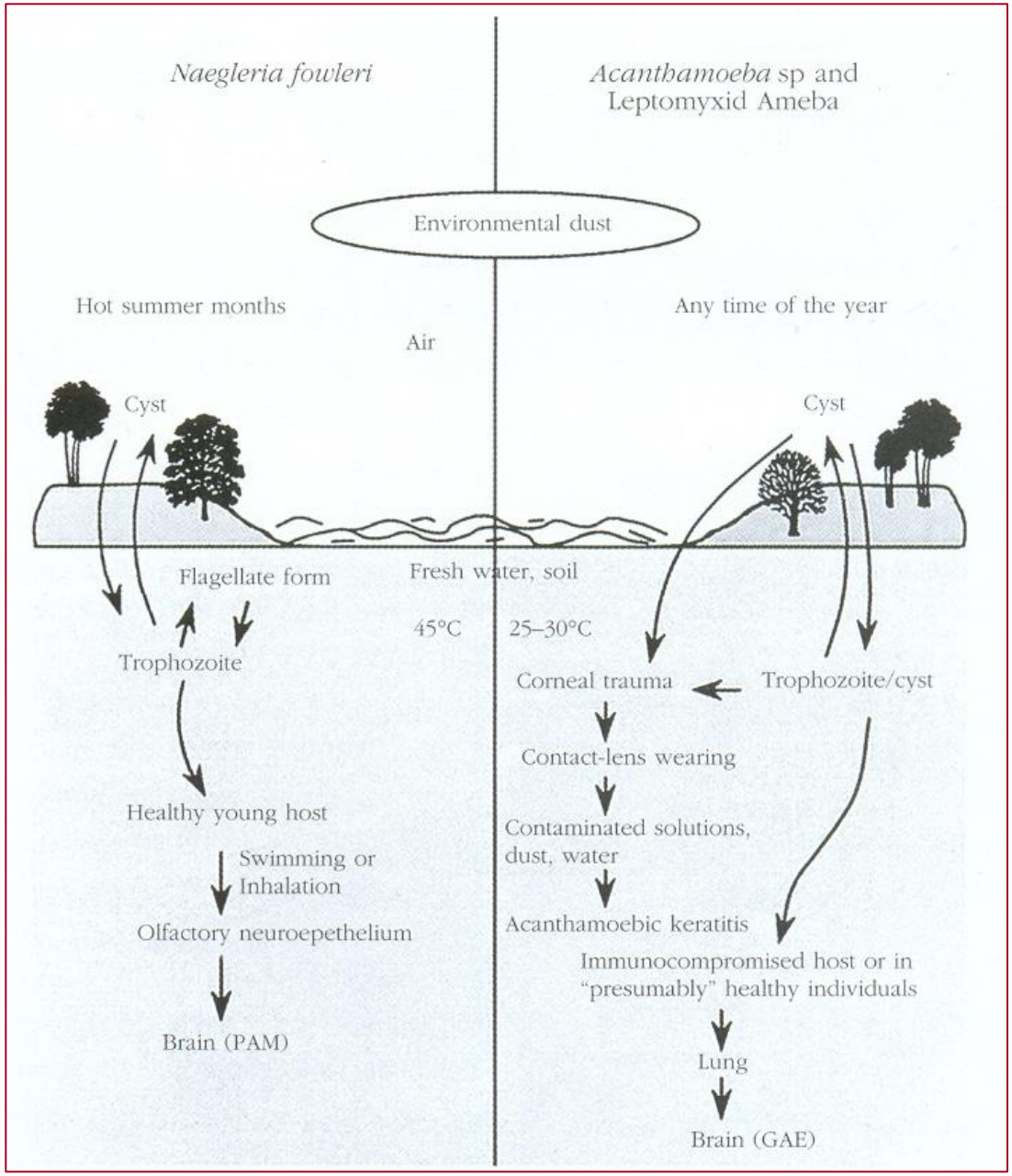
E. dispar

- *E. dispar* is morphologically identical to *E. histolytica*, but does not produce an invasive disease

Free-living amoebas - morphology



Free-living amoebas



Free-living amoebas: *Aacanthamoeba* and *Naegleria* sp.

***Acanthamoeba* sp.**

- found in soil and lakes
- inhabit immunologically privileged sites as eye or brain
- eye infection by contaminated contact lenses

Disease

- ulcerative keratitis – if untreated leads to loss of the eye
- granulomatous amoebic encephalitis (GAE)

Free-living amoebas: *Aacanthamoeba* and *Naegleria* sp.

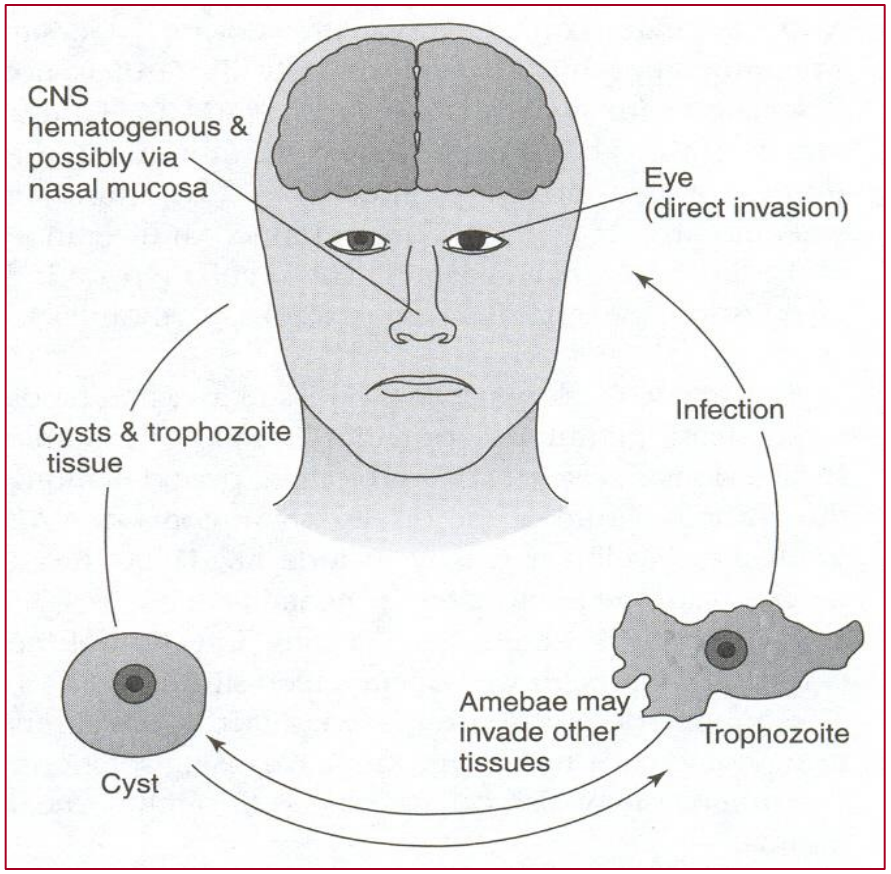
Naegleria fowleri

- in worm water, mud, lakes
- the infection is acquired by accidental inhalation of contaminated water while swimming or playing

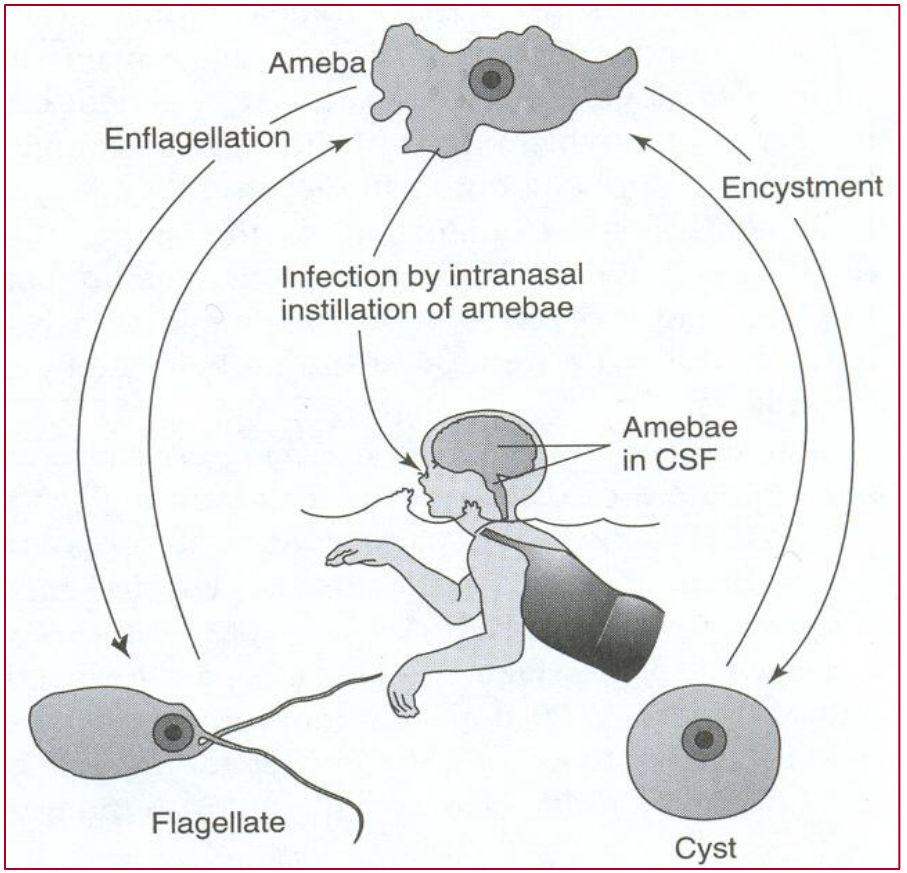
Disease

- primary amoebic meningoencephalitis (PAM) – rapidly turn into a deep coma, almost always fatal

Free-living amoebas – life cycles

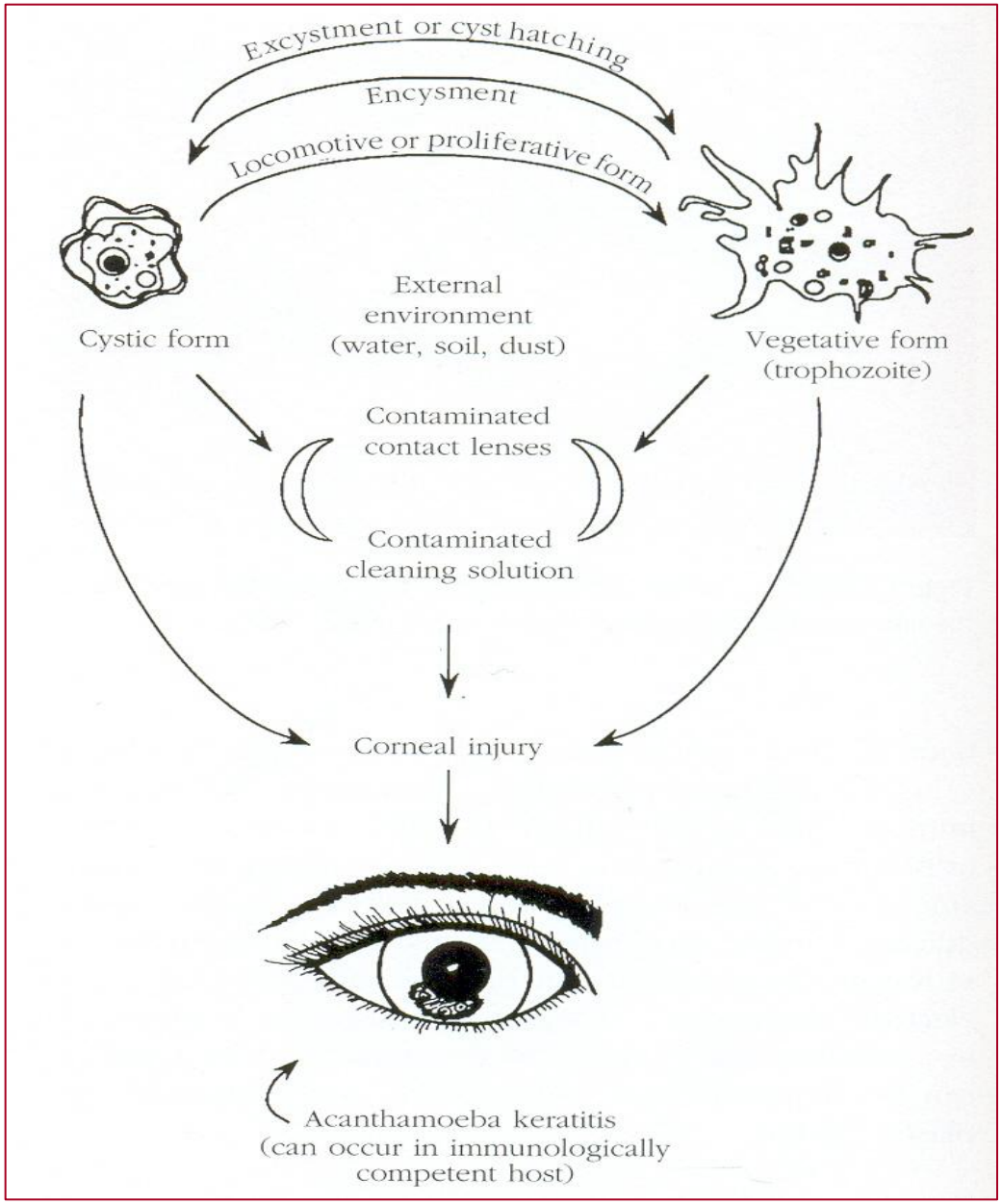


Acanthamoeba spp.

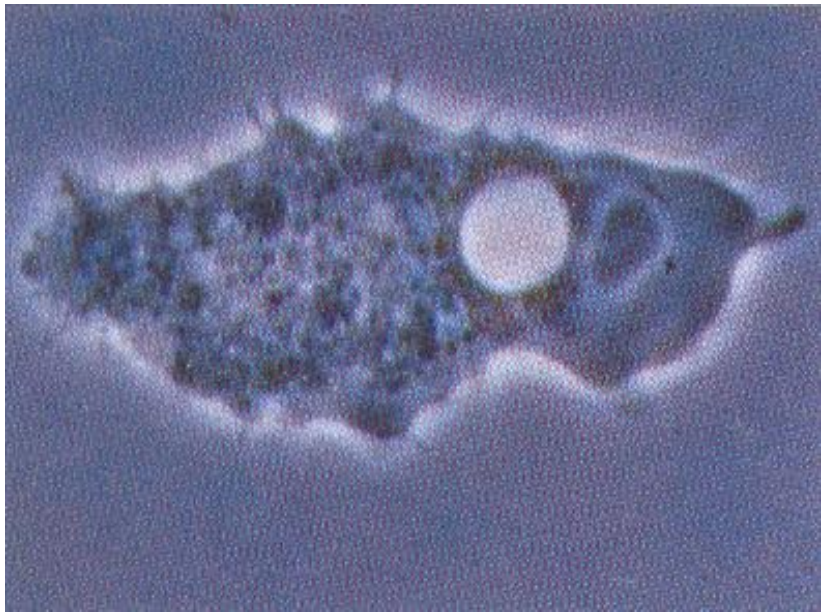


Naegleria fowleri

Acanthamoeba spp. - Keratitis



Acanthamoeba spp. - Keratitis



Acanthamoeba culbertsoni

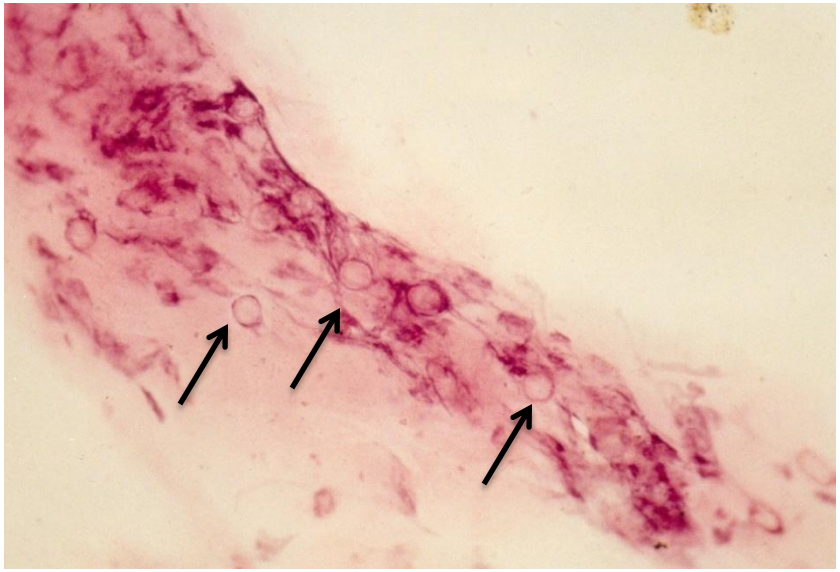
Trophozoite

Eye infection

(*Acanthamoeba* keratitis)

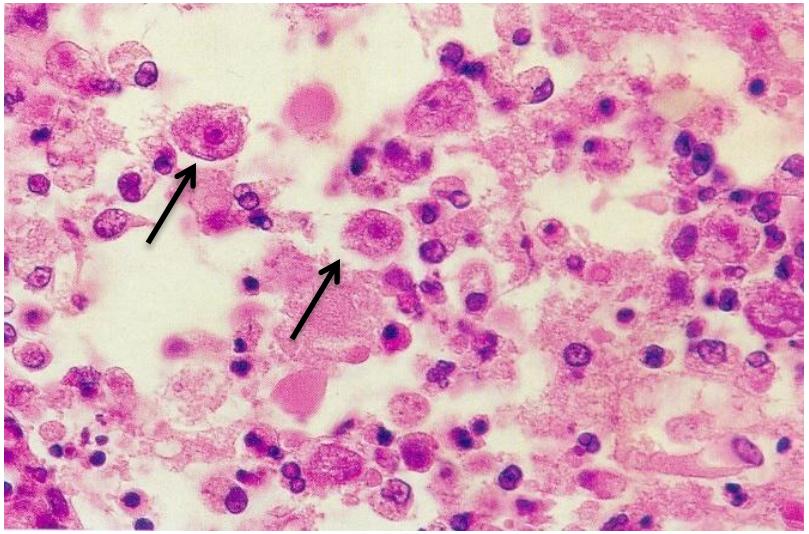
Acanthamoeba spp.

Acanthamoeba spp. - Keratitis



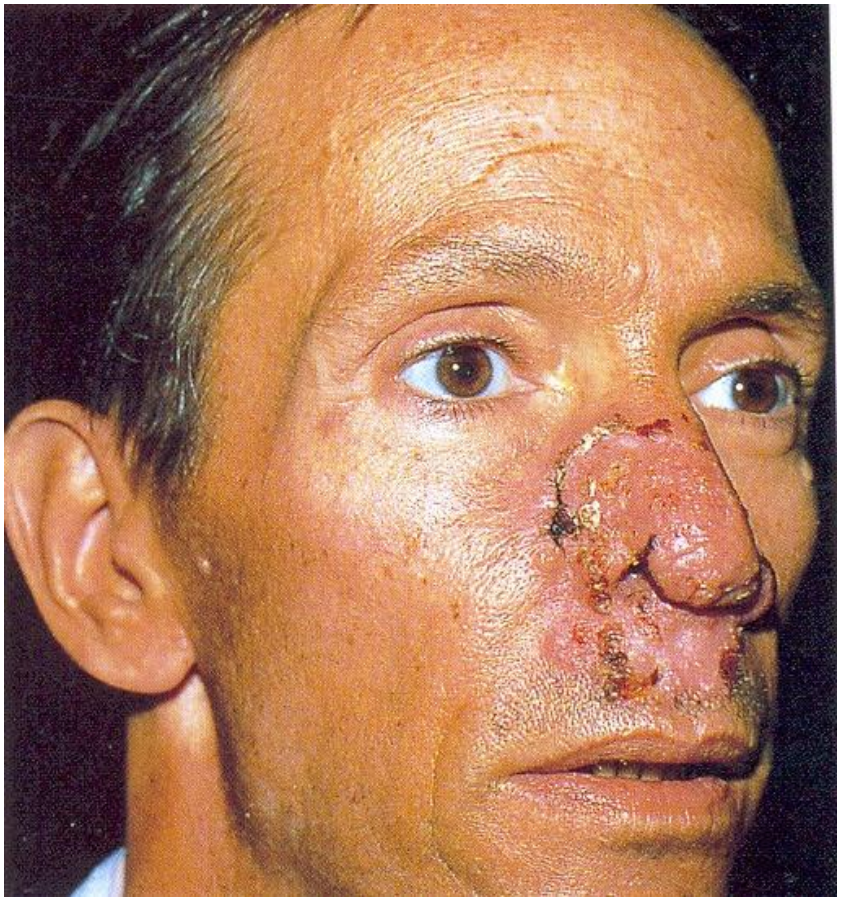
Cysts in corneal screping (H&E)

Acanthamoeba spp. - GAE



Trophozoites in brain (H&E)

Acanthamoeba spp. – skin infection



Naegleria spp.

Naegleria fowleri

trophozoite

N. fowleri in brain (PAM)

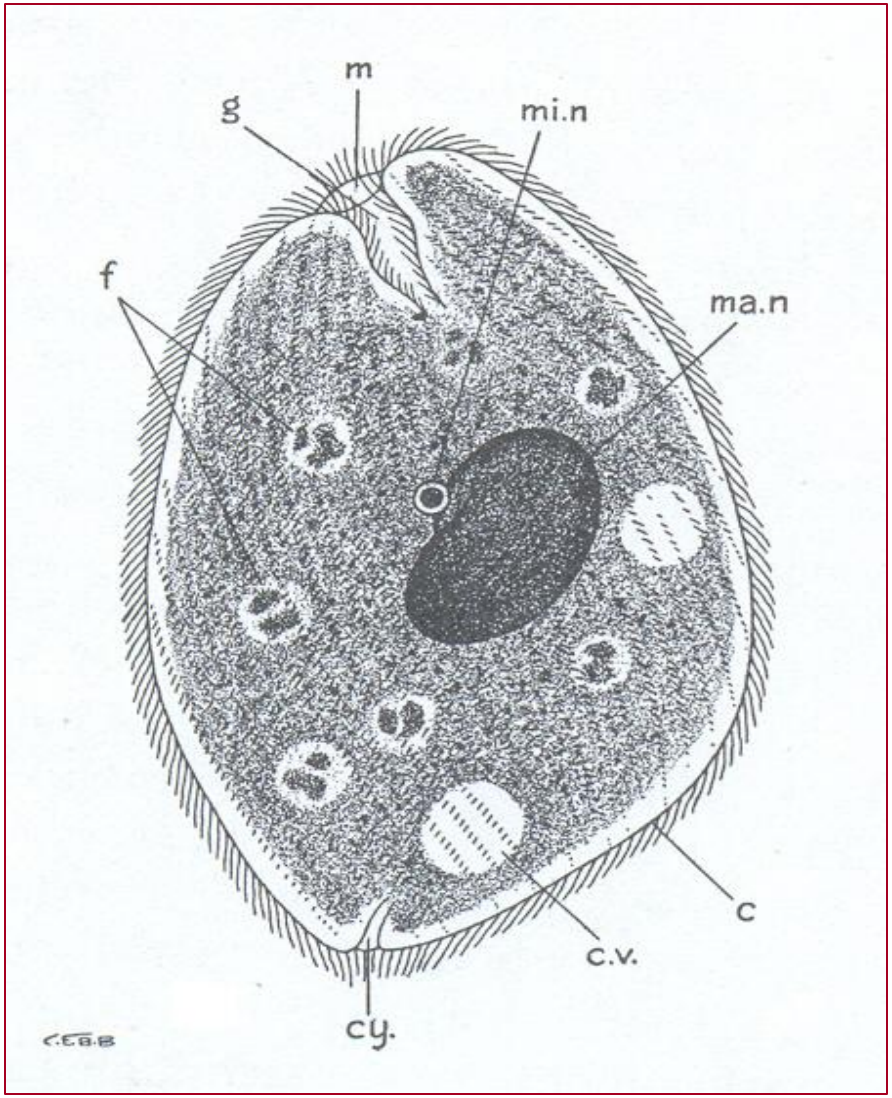
Balantidium coli

- large ciliate, common parasite of pigs, rarely causes disease in humans
- transmission by fecal-oral route

Disease

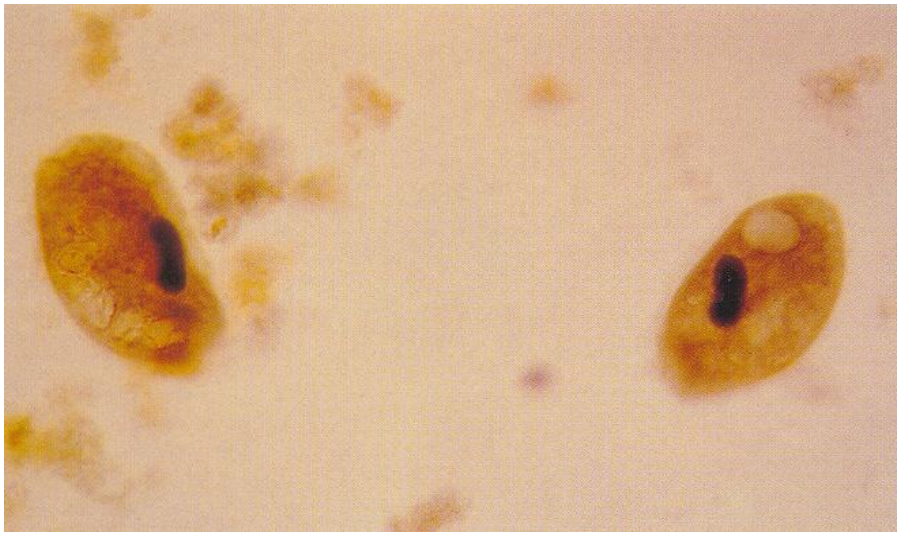
- most infections are asymptomatic
- may develop dysentery and colitis with nausea, vomiting and fever

Balantidium coli – trophozoite

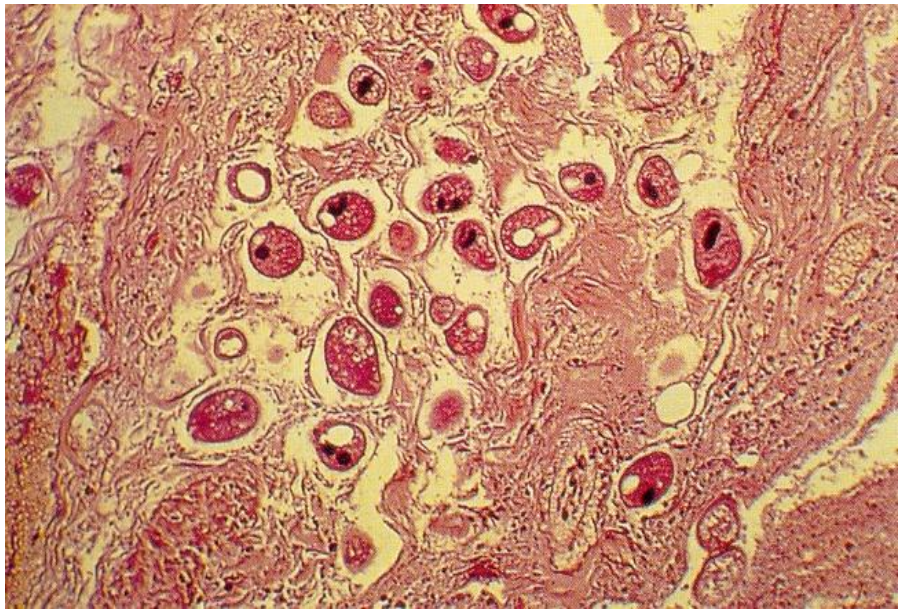


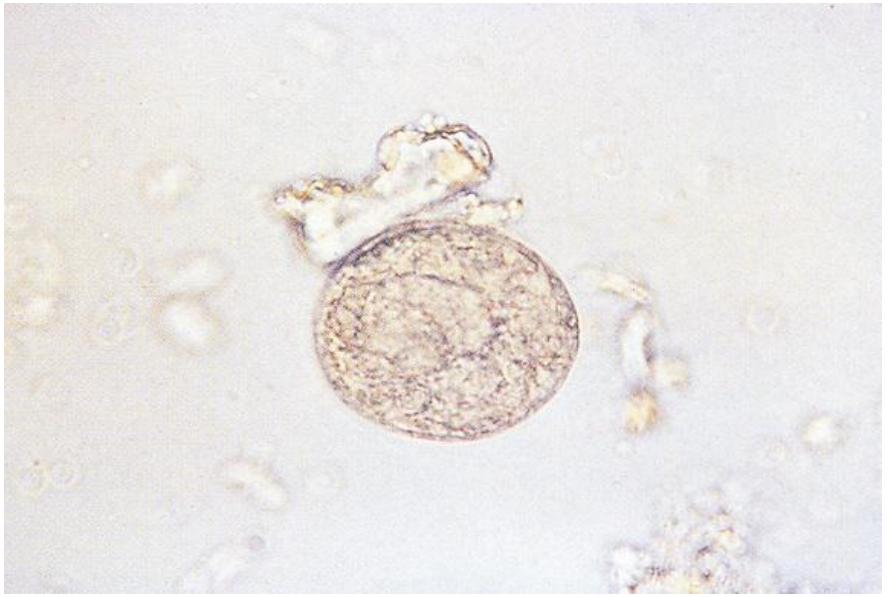
Balantidium coli - diagnosis

Trophozoites in stool specimen



Trophozoites in colon (HP)



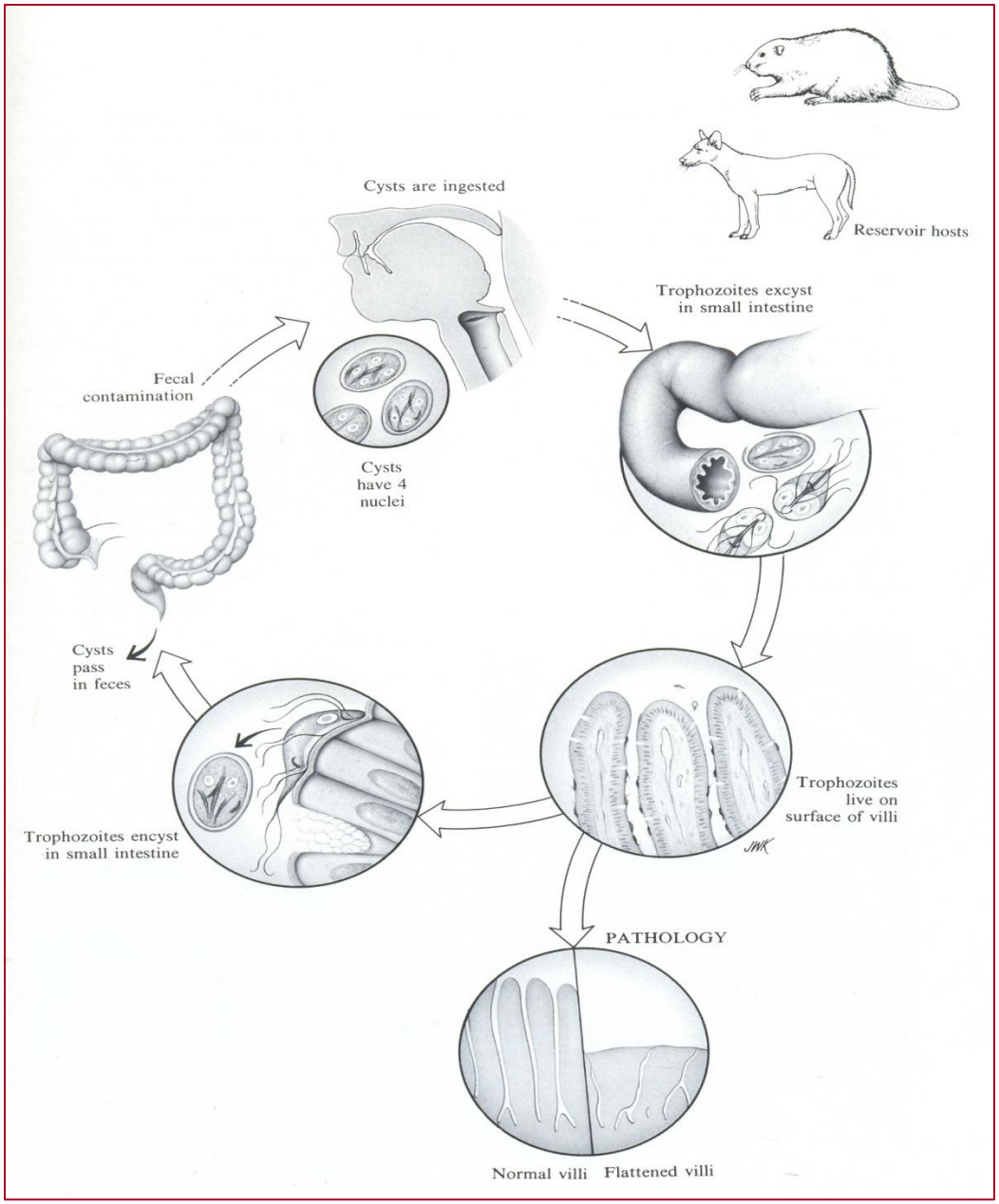


Cyst in stool specimen

Giardia lamblia – etiology

- a flagellate protozoon: *G. lamblia* (*Giardia intestinalis*)
- exists in trophozoite and cyst forms
- the infective form is the cyst of the parasite
- cysts remain infective in water for a few months
- when ingested by a new host, they excyst in the upper gastrointestinal tract and liberate trophozoites, which attach with their suckers to the surface of the duodenal or jejunal mucosa and multiply by binary fission
- when trophozoites drop off the duodenal and jejunal mucosa, they are carried on with the contents in the gut and encyst

Giardia lamblia – life cycle



Giardia lamblia - epidemiology

- globally distributed parasitosis
- infection is usually sporadic and spreads from person to person directly by the fecal-oral route or indirectly by ingestion of fecally contaminated water or food
- humans are the principal reservoir of infection
- overland travelers to the Far East are at high risk for infection

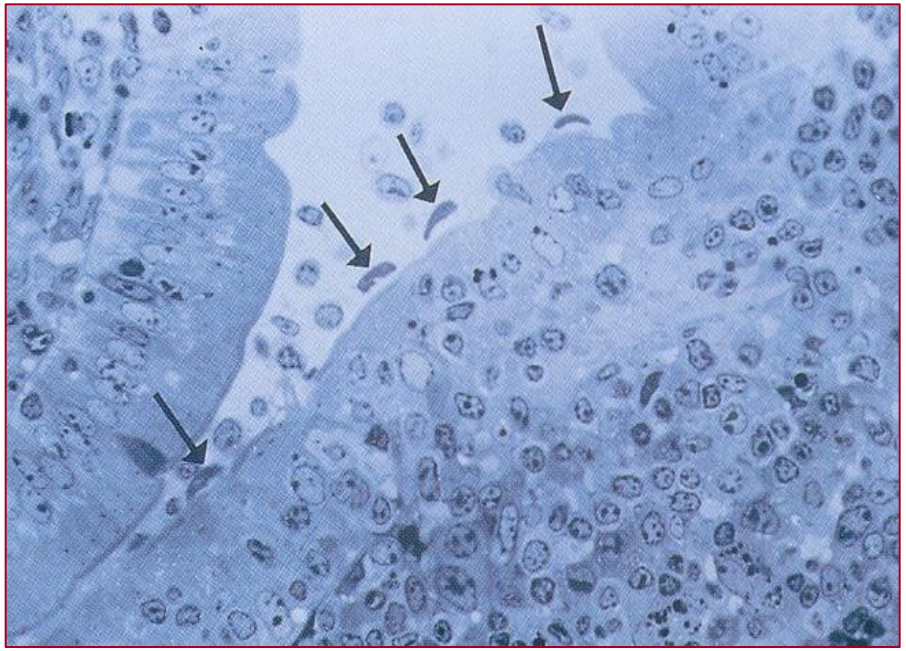
Giardia lamblia – incubation period

- infection may be asymptomatic or symptomatic
- the ratio of asymptomatic to symptomatic cases is high
- children usually acquire the infection but exhibit a high degree of tolerance
- symptoms develop a few days to several weeks (average, 9 days) after ingestion of cysts
- severe infection may develop in immunodeficient hosts
- infection may become chronic

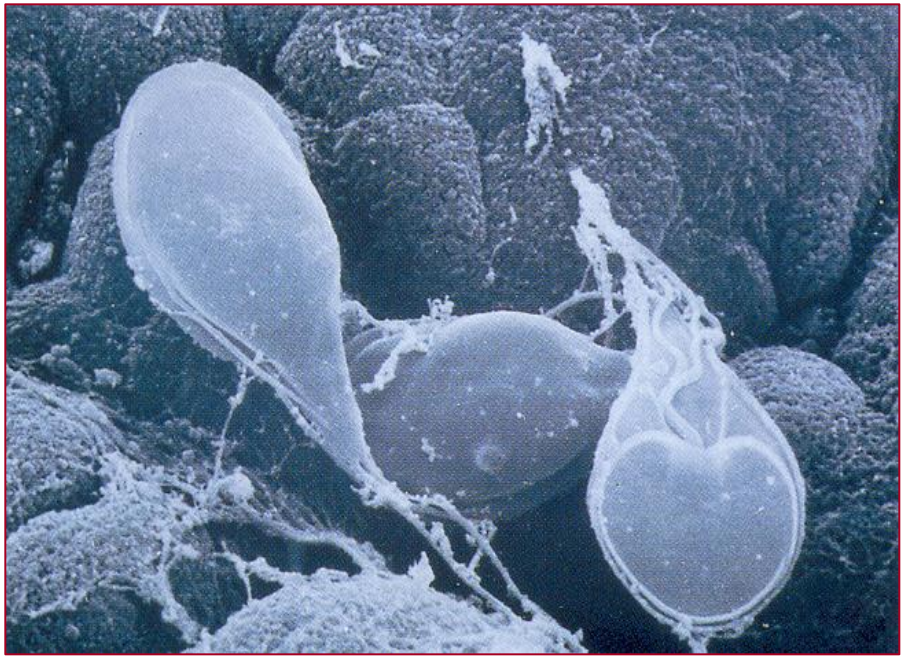
Giardia lamblia – clinical manifestations

- the main symptom is diarrhea (may continue for weeks or months if untreated), steatorrhea
- lead to malabsorption, particularly of lipids and lipid-soluble vitamins (may be difficult in children), loss of weight
- do not penetrate the mucosa
- crampy abdominal pain, urgent call to stool
- stool: pale, offensive, bulky, with much flatus but no blood or mucus
- anorexia and possibly vomiting in each stage of symptomatic infection
- in immunocompetent self-limited infection in 4 weeks

Giardia lamblia



Trophozoites adhere to mucosa



Trophozoites - EM

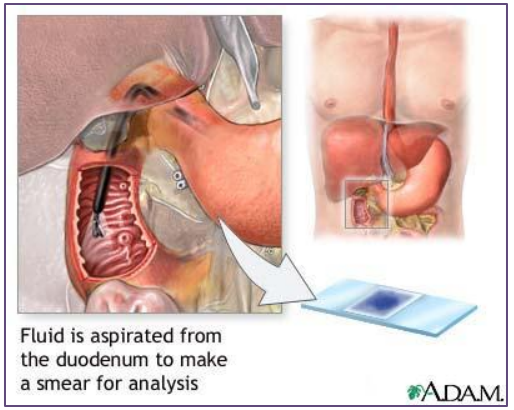
Giardiasis - diagnosis

Duodenal aspiration

- Enterotest ("string test"): a string is taped to the patient's face and a gelatin capsule attached to the string is swallowed. After the capsule has dissolved and the string has reach the duodenum (4 hours later), the string is retrieved and examined for parasites.

Duodenal biopsy

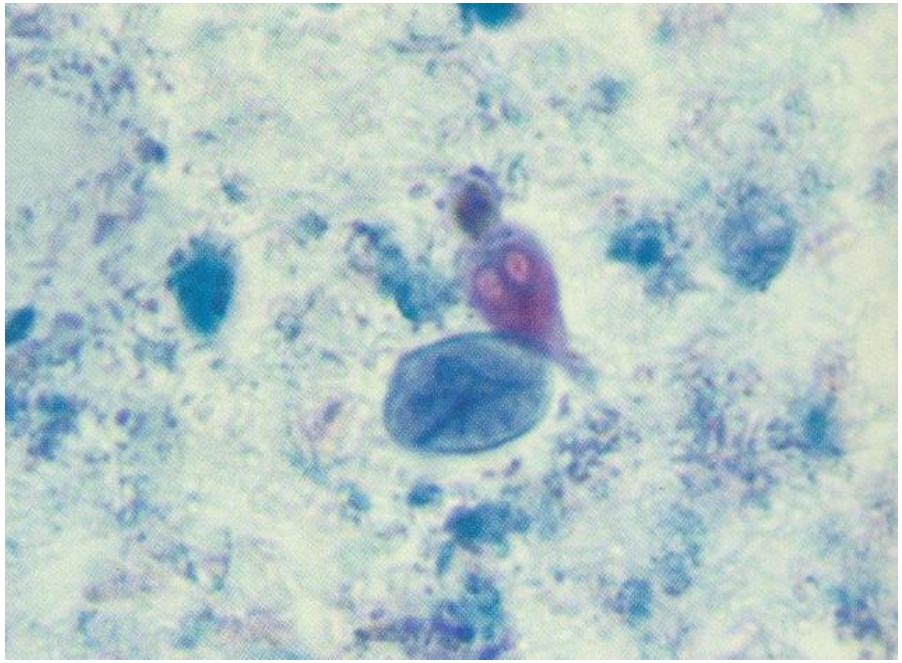
- Intestinal biopsy reveals partial villous atrophy
- trophozoites may be seen on the surface of the bowel



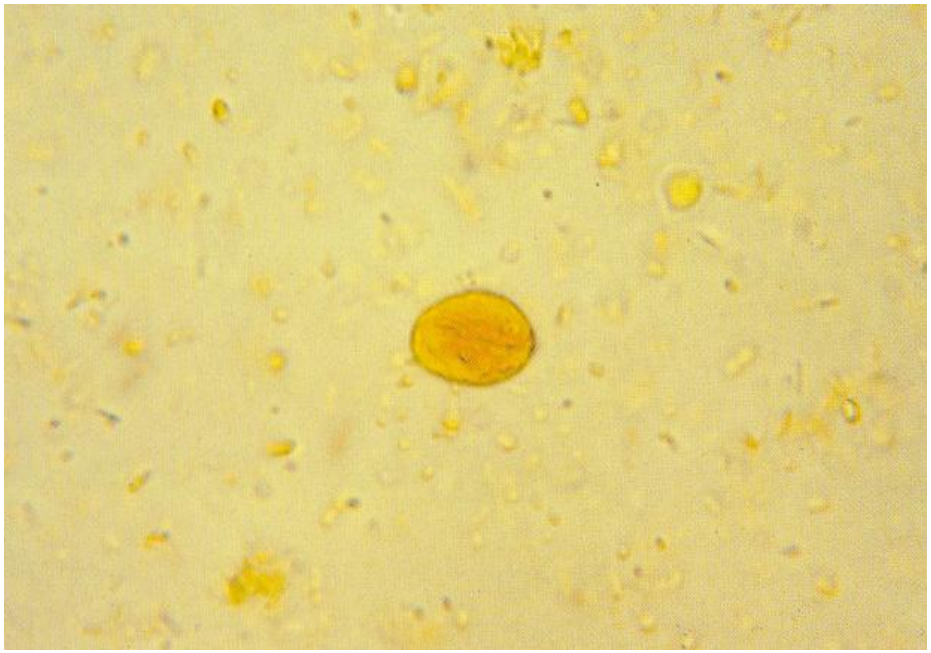
Giardiasis - diagnosis

- direct saline smear of stool for characteristic cysts
- repeat three times for up to 90% success of identifying the cysts versus 50% to 70% on single stool specimen examination
- trophozoites are found in fresh diarrheal stools
- trophozoite: pear-shaped, 15 µm long, 9 µm wide, 3 µm thick; possesses four pairs of flagella
- cysts are found in form stools
- cyst: oval, 8 to 14 µm long, 5 to 10 µm wide; contains four small nuclei and a central refractile axostyle

Giardia lamblia



Trophozoite in stool specimen



Cyst in stool specimen – wet mount

Giardiasis - diagnosis

Indirect diagnosis

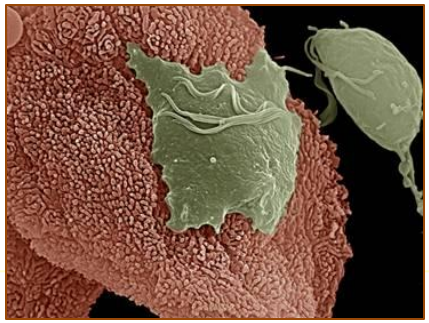
- serology as useful diagnostic aids
- antigen-capture ELISA can be used to demonstrate submicroscopic infections in faeces
- ELISA to detect IgM in serum provides evidence of current infection
- IgA-based ELISA can detect specific antibodies in saliva

Giardiasis - treatment

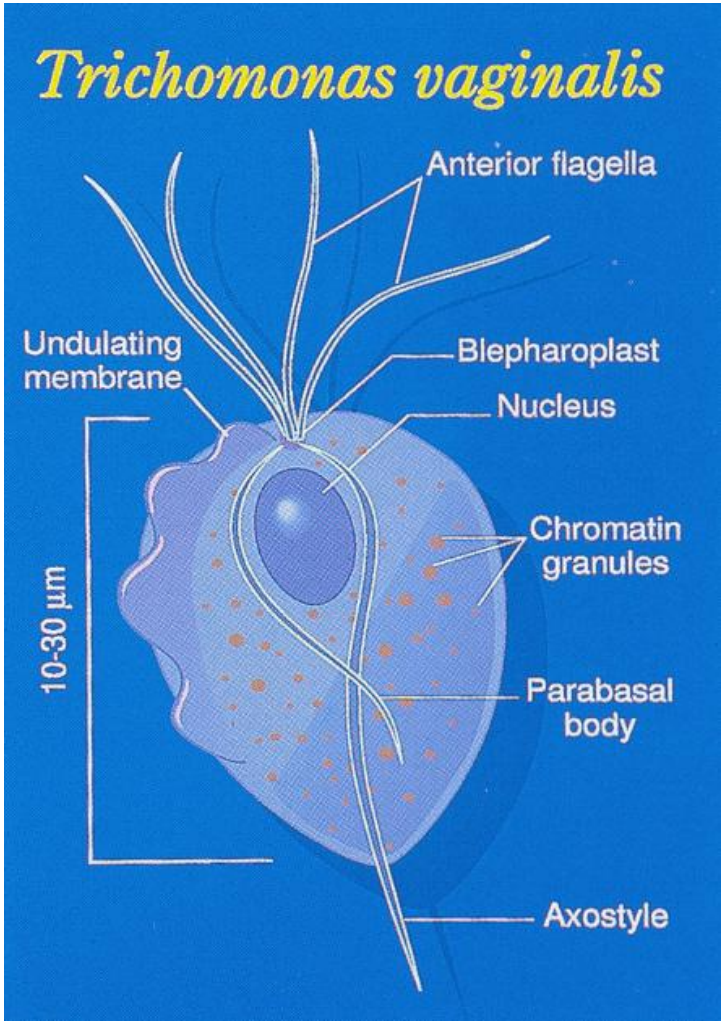
- Metronidazole (with efficacy up to 80%-95%)
  - in adults: either 250 to 500 mg for 5 days
  - avoid alcohol intake, as it may produce side effects such as headache and flushing.
  - in children, dosage modified: 5 mg/kg for 7 days

Trichomonas vaginalis

- ✓ **Exist only as a trophozoite!!!** (no cyst form)
- ✓ Cosmopolitan, strictly human, sexually transmitted flagellate
- ✓ Disease: trichomoniasis



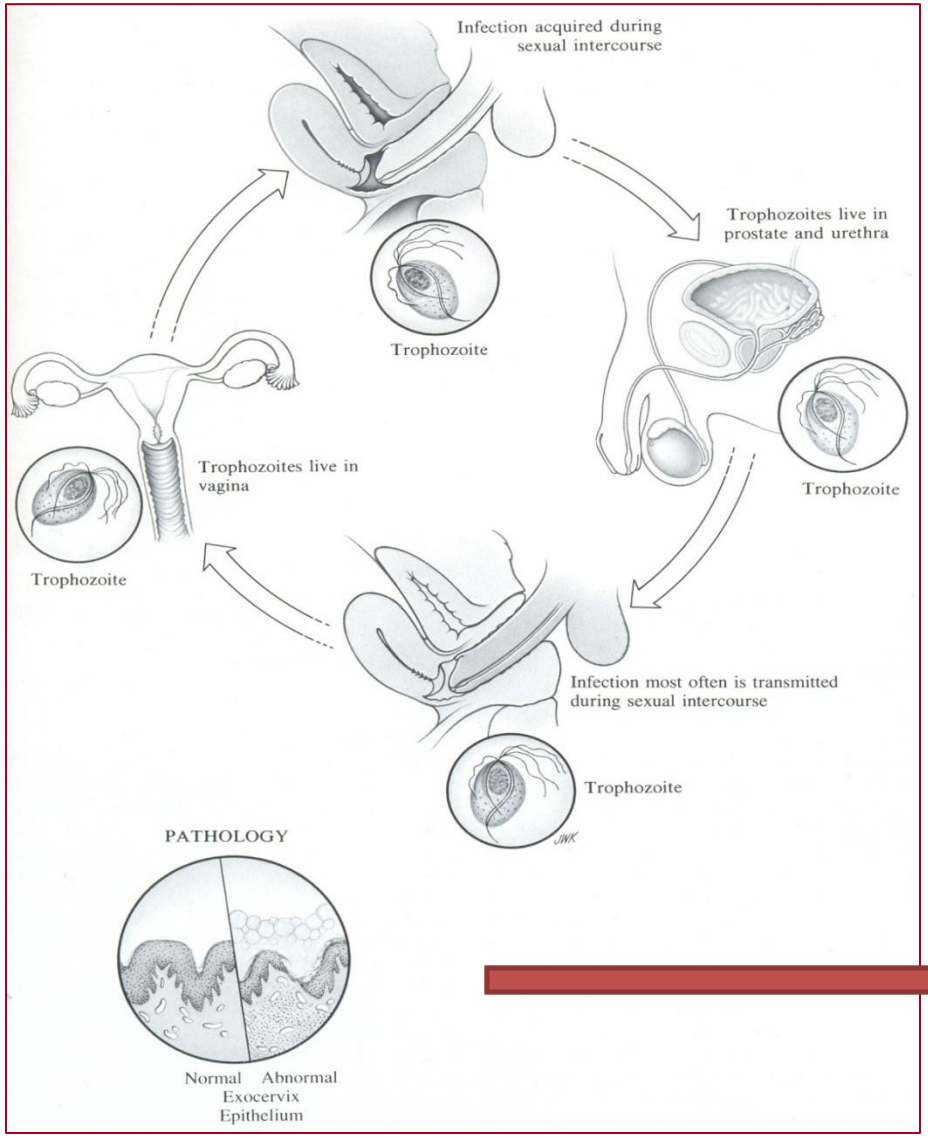
Trophoyoite adhere to epithelial cells



Trichomonas vaginalis – *e*pidemiology

- Trichomoniasis is a sexually transmitted disease that accounts for 25% of vaginitis
- usually women have symptoms, while males are asymptomatic but may act as reservoirs of infection
- 30% women will develop symptoms within 6 months
- *T. vaginalis* is isolated from prostatic secretions of 70% of male consorts of infected women

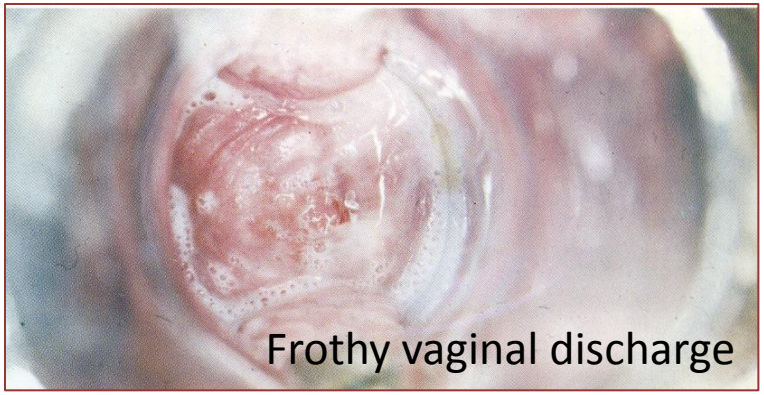
Trichomonas vaginalis – life cycle



Trichomonas vaginalis – clinical manifestations

Females: vulvar erythema, pruritus, edema

- Vaginal discharge
  - Purulent: 60%
  - Frothy: 10% to 35%
  - Gray: 45%
  - Yellow-green: 35%


Frothy vaginal discharge

- Strawberry or “flea-bitten” cervix, which can be seen by colposcopy

Males: asymptomatic, urethral discharge, dysuria

Trichomonas vaginalis – diagnosis

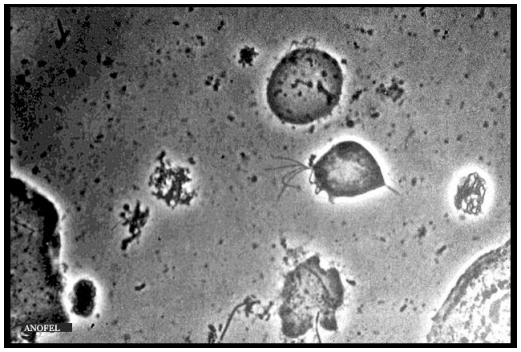
Specimen: vaginal secretions,
urethral discharge,
urine sediment,
prostatic secretions

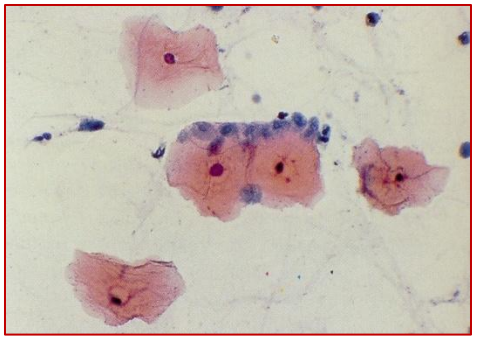
Methods:

- Microscopic examination
  -direct wet mount
  -Giemsa stain
  -Acridine orange fluorescent stain

- Culture – Diamond's medium
- PCR

Findings: trophozoite!!!





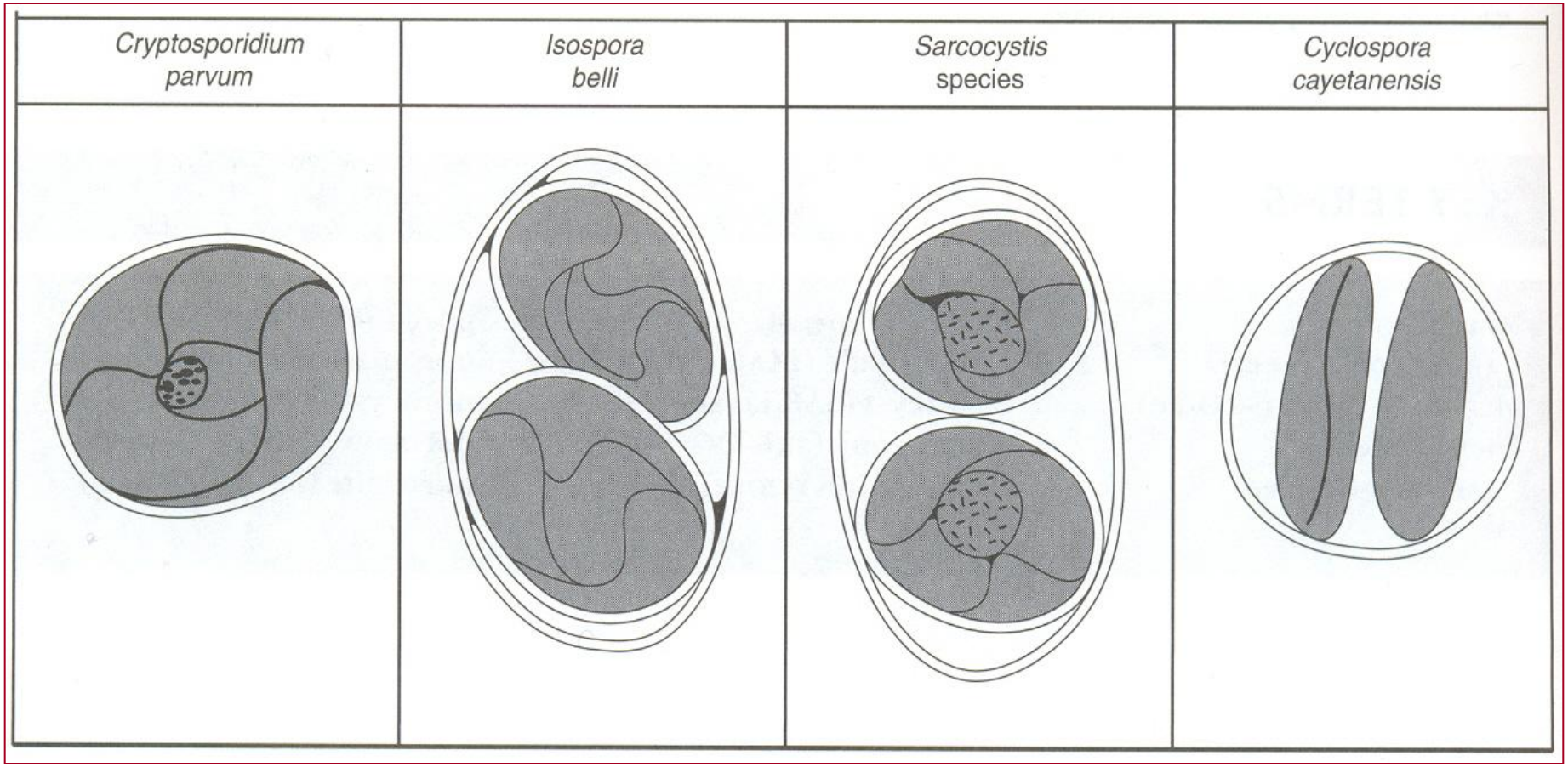
Trichomoniasis – diagnosis

- examination of a wet saline mount of vaginal discharge under a microscope shows motile, flagellated protozoa in a background of many polymorphonuclear leukocytes
- the pH of vaginal discharge is greater than 4.5
- culture for *Trichomonas* in special medium (Diamond medium) has a high yield of positives
- direct examination and culture of urine sediment is the test of choice for diagnosing males

Trichomoniasis – treatment

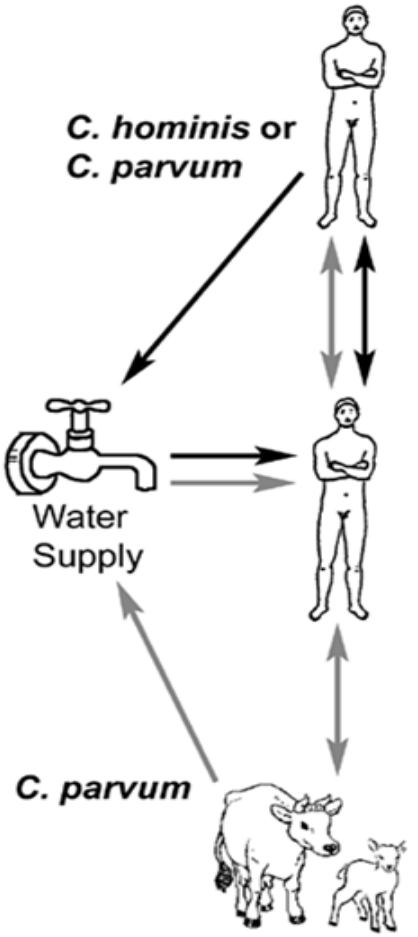
- the preferred treatment is a single 2-g dose of metronidazole. Alternately, 500 mg bid for 7 days can be used
- coitus should be avoided until treatment is complete and both partners are asymptomatic
- a single dose of 2 g of metronidazole may be given to pregnant women only after the first trimester
- treatment failure occurs in up to 30% when the male partner is not treated

Intestinal Coccidia: oocyst morphology



Cryptosporidium parvum

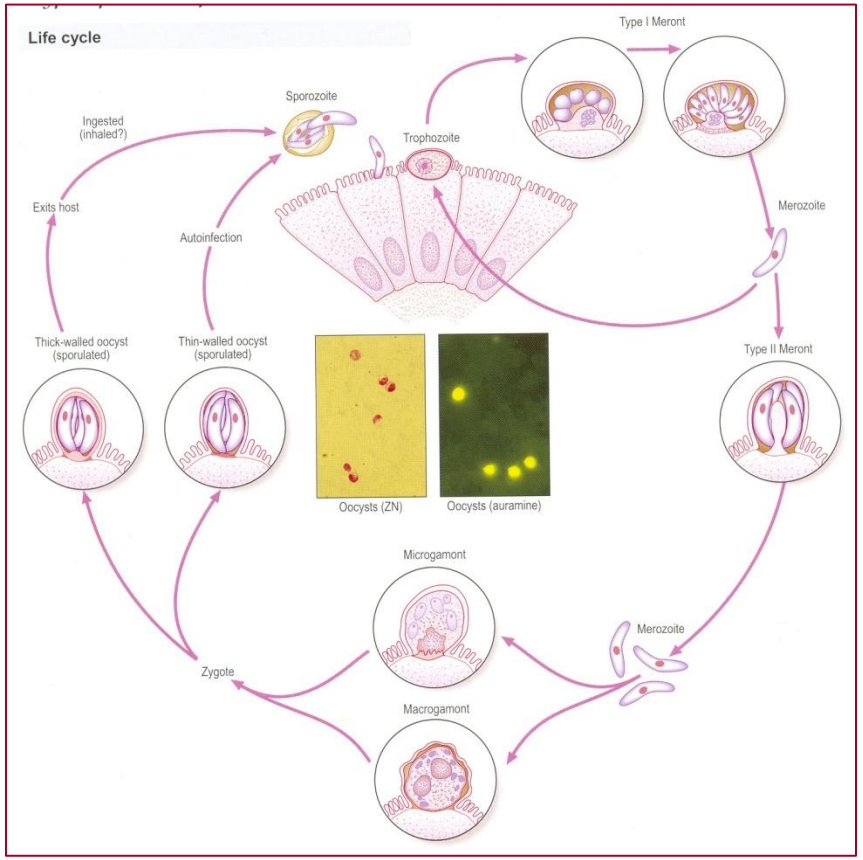
- infection usually occurs by ingestion of oocysts from fecally contaminated water
- oocysts can survive as long as 18 months in the environment
- studies have shown that ingestion of less than 1,000 oocysts can lead to disease



Cryptosporidium parvum

- intracellular protozoan that is responsible for self-limited diarrhea in children and adults and protracted and even fatal diarrhea in patients with HIV infection

- the entire life cycle occurs within one person



Cryptosporidium parvum

Incubation

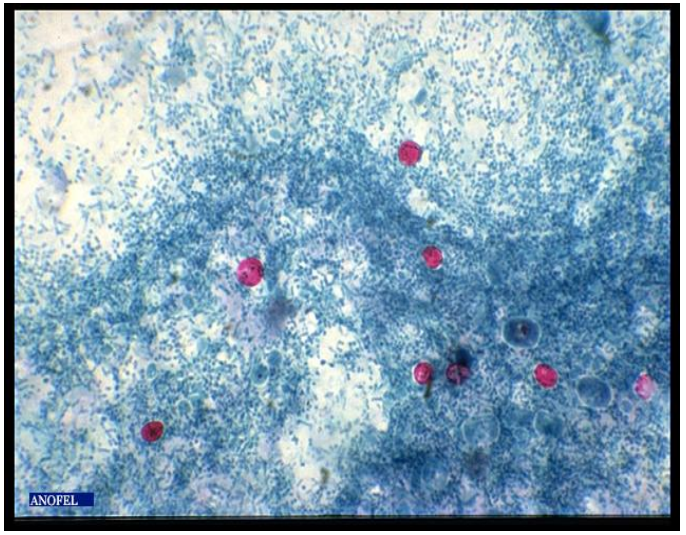
- Incubation is between 7 and 10 days

Clinical Manifestations

- diarrhea in normal persons occurs at various degrees of severity from 2 days to 1 month
- patients may have crampy abdominal pains
- low-grade fevers may occur
- in patients with immunosuppression, such as HIV infection, voluminous diarrhea with as much as 15 L/d can occur

Cryptosporidiosis - diagnosis

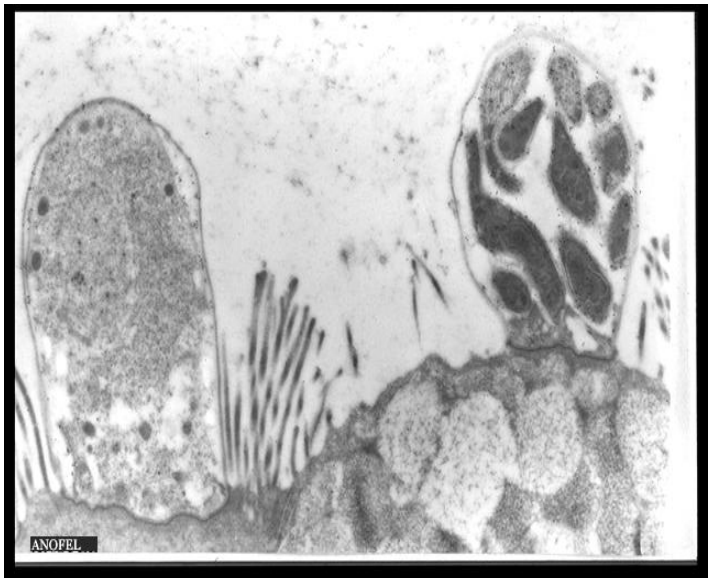
- stool specimens reveal oocysts with Giemsa stains or modified acid-fast stains
- fluorescent antibody stains for stool or tissue specimens are available
- fecal leukocytes are not present
- fat absorption is impaired
- vitamin B12 levels may become low



Modified Ziehl-Neelsen stain

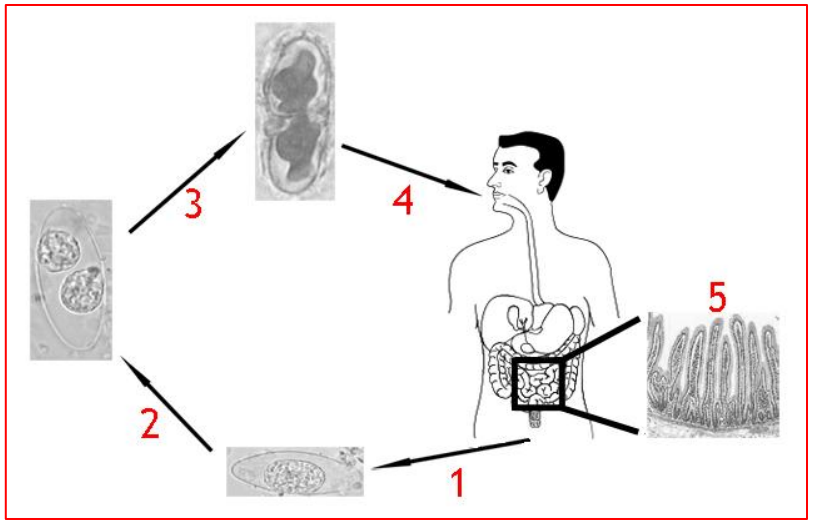
Cryptosporidiosis - treatment

- there is no effective treatment for this illness
- patients who are immunocompetent are likely to run a self-limited illness of several days to 6 weeks, for which supportive care is given
- in HIV-infected individuals, supportive care is critical



Isospora belli – life cycle

- 1 Immature, unsporulated oocyst is excreted through feces.
- 2 Sporoblast divides into two.
- 3 Each sporoblast develops into a sporocyst with 4 sporozoites, resulting in mature oocysts.
- The time spent in stages 1 through 3 is 2-3 days.
- 4 Mature oocyst is ingested.
- 5 Oocyst bursts. Sporozoites are released and lodge into the intestinal lining. Sporozoites undergo asexual reproduction to form merozoites. The merozoites mature into gametes which undergo fertilization to produce a new oocyst.

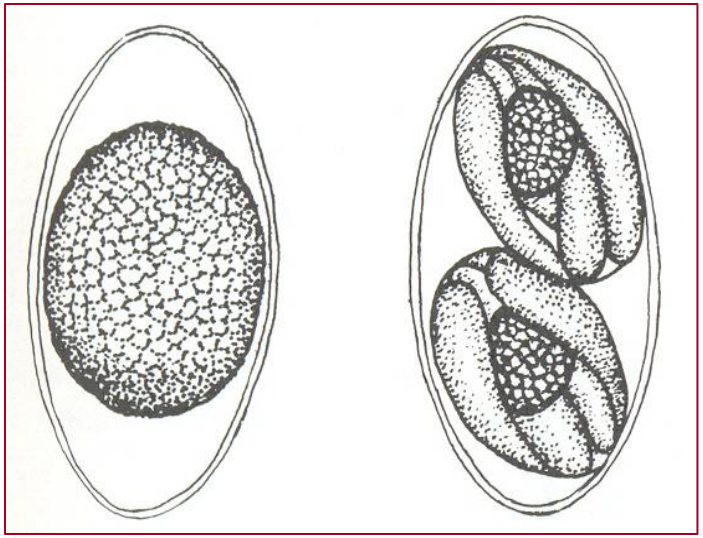


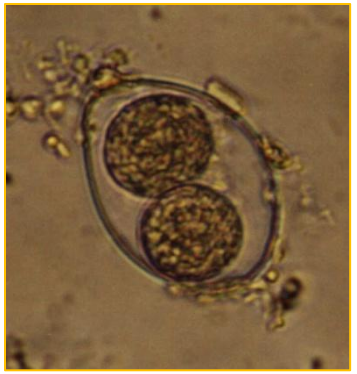
Isospora belli

Isosporiasis :

diarrhea, malabsorption, eosinophilia,

particularly in patients with AIDS



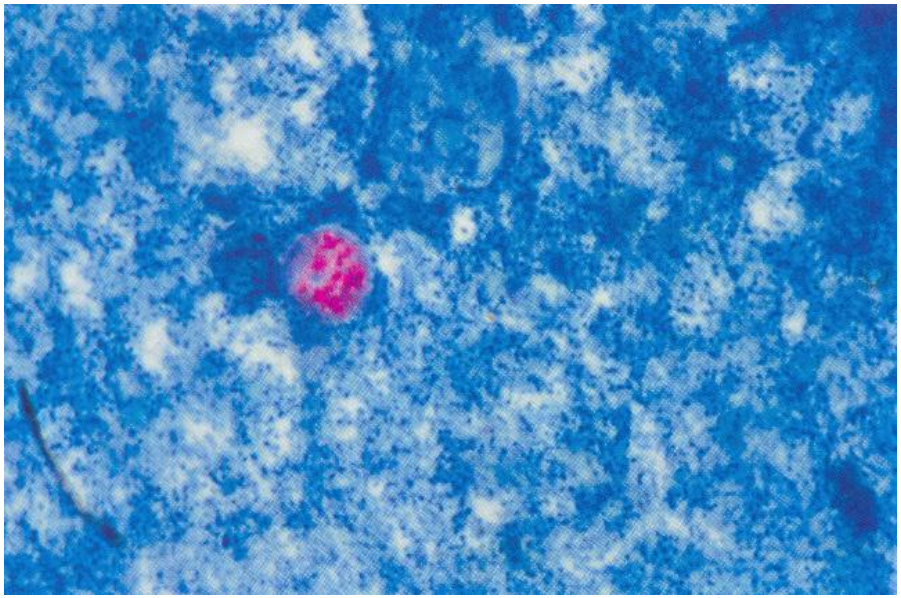


Diagnosis:

- examination of concentrated stools
- Kinyoun stain

Cyclospora cayetanensis

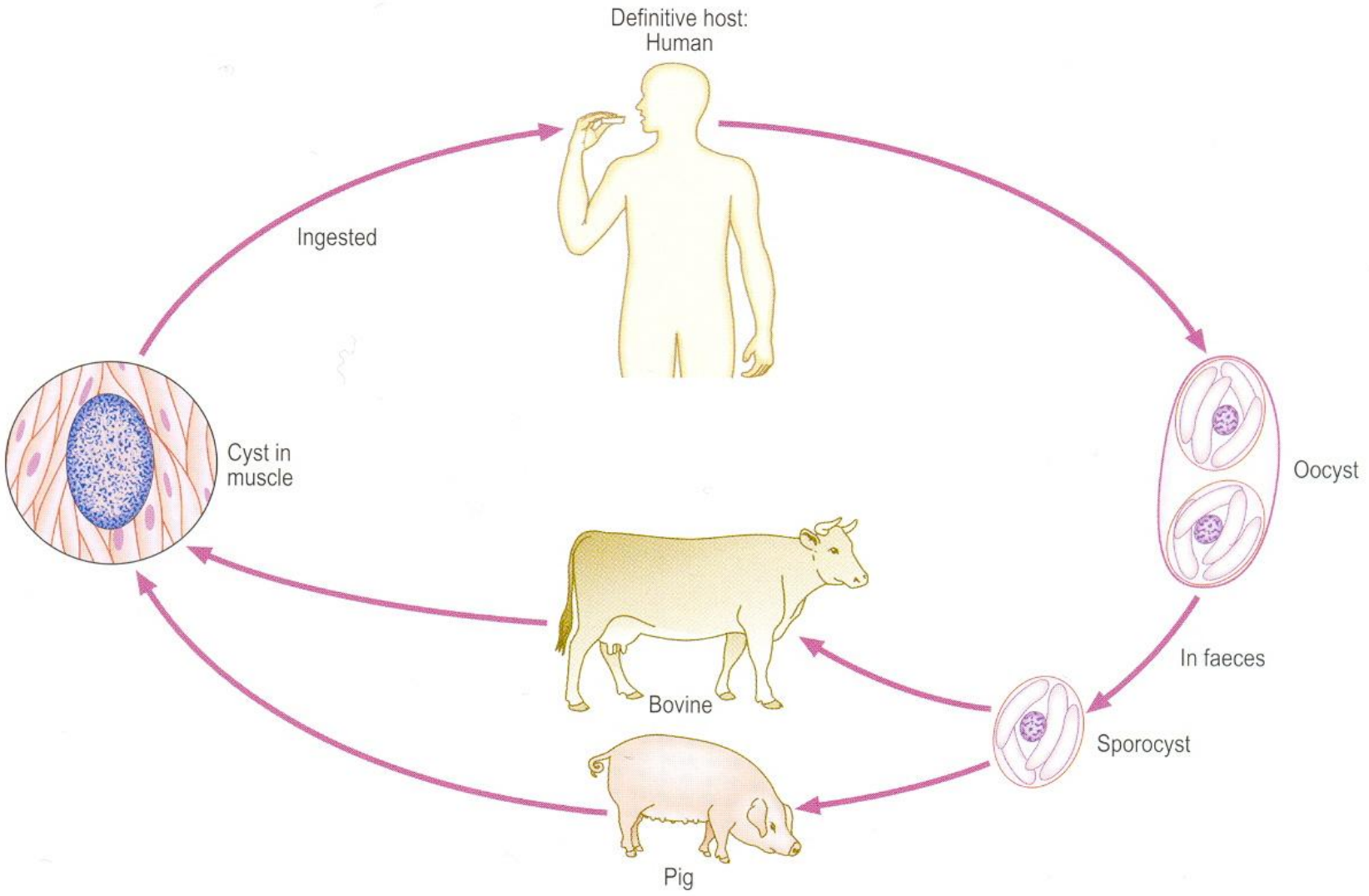
oocysts – stool sample, wet mount



Oocyst – stool sample, stain preparation (mZN)

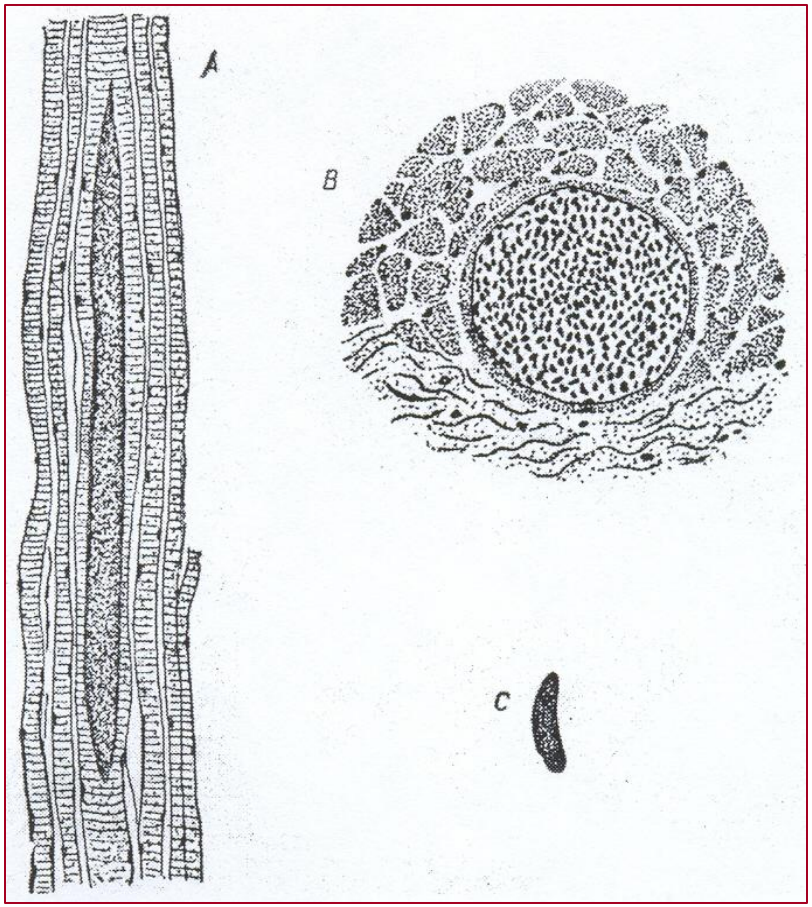
- Epidemiology: contaminated water, fruits and vegetables
- Manifestation: diarrhea
- Diagnosis: oocysts in stool samples
- Treatment: Bactrim®

Sarcocystis spp. – life cycle

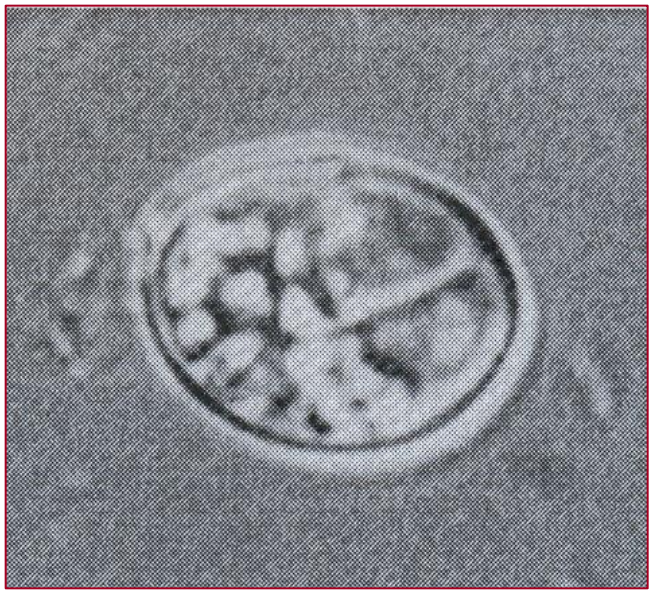


Occasionally humans can act as intermediate hosts for *Sarcocystis* of other animals.

Sarcocystis spp.:
S. suihominis and *S. bovihominis*



Miescher's tubes



Sarcocystis suihominis

(oocyst)

Unclassified protozoa ***Blastocystis hominis***

Large central body (vacuole)

•worldwide
• commonly found in stool specimens
• pathogenicity is unclear (not to cause any disease in most cases of isolation)
• self-limited, acute diarrhea
Diagnosis: identification in stool specimen