



## Protozoa

### General Features

- ✓ Single-celled eukaryotic microorganisms belonging to the kingdom Protista are classified as Protozoa
  - ✓ The single protozoal cell performs all functions.
  - ✓ Most of the protozoa are completely nonpathogenic, but a few may cause major diseases such as malaria, leishmaniasis, and sleeping sickness.
  - ✓ Protozoa like *Toxoplasma gondii*
  - ✓ Trophozoites are the active feeding and growing stage of protozoa.
- Cysts are the resting or resistant stage of protozoa, bounded by a tough cell wall.
  - Protozoa multiply by both asexual and sexual modes of reproduction.

### Classification of Protozoa

Protozoan parasites of medical importance have been classified within the kingdom Protista, subkingdom Protozoa, which is further divided into four phyla.

#### 1- Amoebae

These protan animalcules can assume any shape and crawl along surfaces by means of foot-like projections called pseudopodia (literally meaning false feet).

#### 2- Flagellates

These protozoa have whip-like appendages called flagella as the organs of locomotion.

#### 3- Phylum Apicomplexa

Phylum Apicomplexa was formerly known as Sporozoa. Members of this group possess, at some stage in their life cycle, a structure called the apical complex serving as the organ of attachment to host cells. To this group belong the malarial parasites (suborder: Family: Plasmodiidae), *Toxoplasma*

#### 4- Phylum Ciliophora

These protozoa are motile by means of cilia, which cover their entire body surface. The only human parasite in this group is *Balantidium coli*, which rarely causes dysentery.

## Amoebae

Amoebae are structurally simple **protozoans** that have no fixed shape. Pseudopodia are formed by the amoeba by thrusting out ectoplasm, followed by endoplasm.

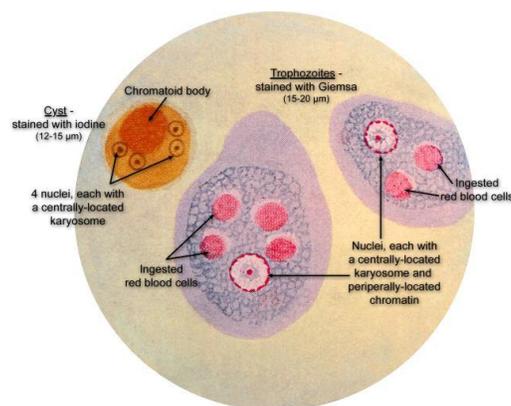
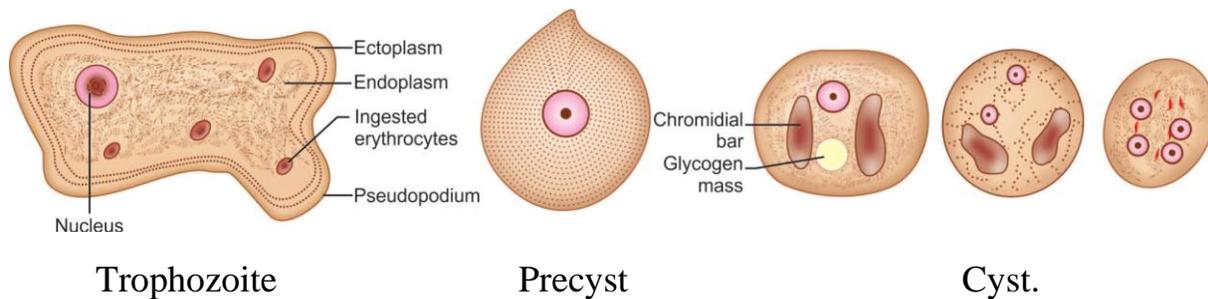
### *Entamoeba Histolytica:*

*E. histolytica* is worldwide in prevalence, being much more common in the **tropics than elsewhere**. It has been found wherever sanitation is poor, across all climatic zones from Alaska (61°N) to the Strait of Magellan (52°S).

### Morphology

*E. histolytica* occurs in 3 forms

- Trophozoite
- Precyst
- Cyst.





## Trophozoite

Trophozoite is the **vegetative or growing stage of the parasite** (Fig. 1A). It is the only **form present in tissues**. It is irregularly shaped and ranges in size from 12–60  $\mu\text{m}$ , with an average of 20  $\mu\text{m}$ .

It is large and actively motile in freshly passed dysenteric stool, whereas it is smaller in convalescents and carriers. **Pseudopodia are finger-like projections formed** by sudden jerky movements of ectoplasm in one direction, followed by the streaming in of the whole endoplasm.

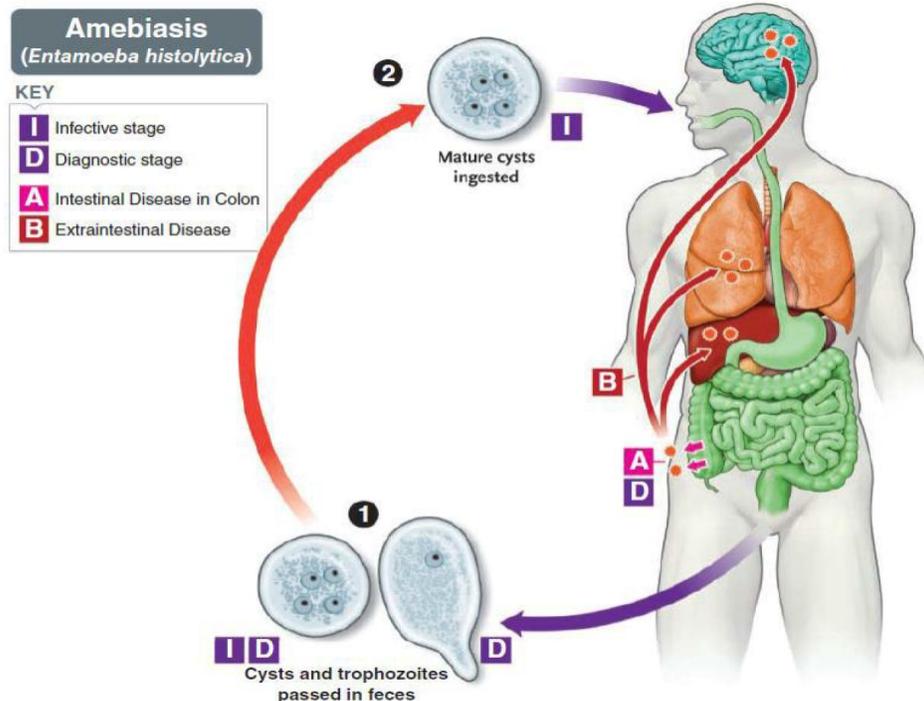
## Cysts Stage

Cysts survive outside the host in **water and soils and on foods**, especially under moist conditions on the latter. When swallowed, they cause infections by excysting (to the troph. stage) in the digestive tract.

## life cycle

E. histolytica passes its life cycle only in 1 host-man

- ✓ Infective form: **Mature quadrinucleate** cyst (**has four nuclei**) passed in the feces of convalescents and carriers.
- ✓ The cysts can remain viable under moist conditions for about 10 days.
- ✓ Mode of transmission: Man acquires infection by swallowing food and water contaminated with cysts.
- ✓ Excystation: When the cyst reaches **the caecum or the lower part of the ileum**, due to the alkaline medium, the cyst wall is damaged, leading to **excystation**.
- ✓ Metacystic trophozoites: The nuclei in the metacyst immediately undergo division to **form 8 nuclei**, each of which gets surrounded by its own cytoplasm to become **8 small amoebulae** or **metacystic trophozoites**.
- ✓ If excystation takes place in the small intestine, the metacystic trophozoites do not colonize there, but are carried to **the caecum**.
- ✓ The optimal habitat for the metacystic trophozoite is the **submucosal tissue of the caecum and colon**, where they lodge in the glandular crypts and grow by binary fission.
- ✓ Some develop into **precystic forms and cysts**, which are passed in feces to repeat the cycle.



## Pathogenesis and Clinical Features

*E. histolytica* causes intestinal and extraintestinal amoebiasis.

- The incubation period is highly variable. On average, it ranges from 4 days to 4 months.

### Intestinal Amoebiasis

- The lumen-dwelling amoebae do not cause any illnesses. They cause disease only when they invade the intestinal tissues.
- The metacystic trophozoites penetrate the columnar epithelial cells in the colon.
- Mucosal penetration by the amoeba produces discrete ulcers with a pinhead center and raised edges
- Ulcers appear initially on the mucosa as raised nodules with pouting edges. They later break down, discharging brownish necrotic material containing large numbers of trophozoites.

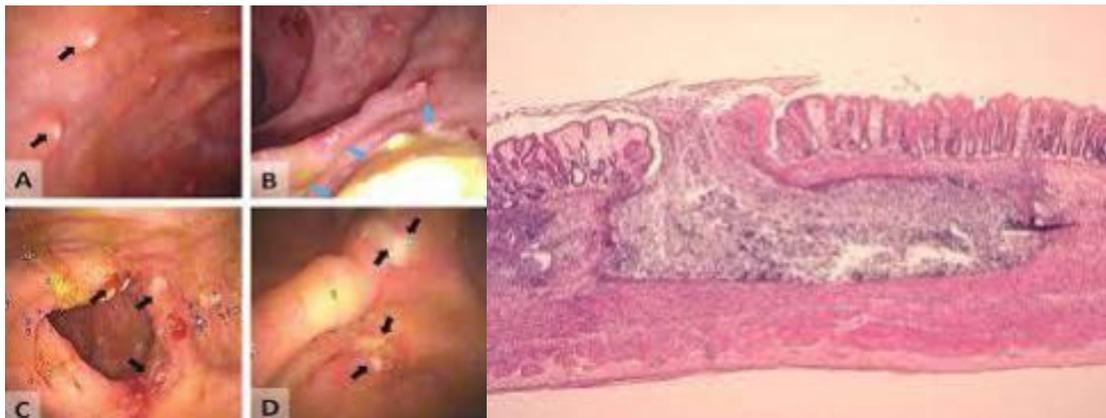


- The typical amoebic ulcer is flask-shaped in cross section, with the mouth and neck being narrow and the base large and rounded.

### Signs and symptoms of amoebic dysentery:

In severe cases of intestinal amoebiasis, the organism invades the lining of the intestine,

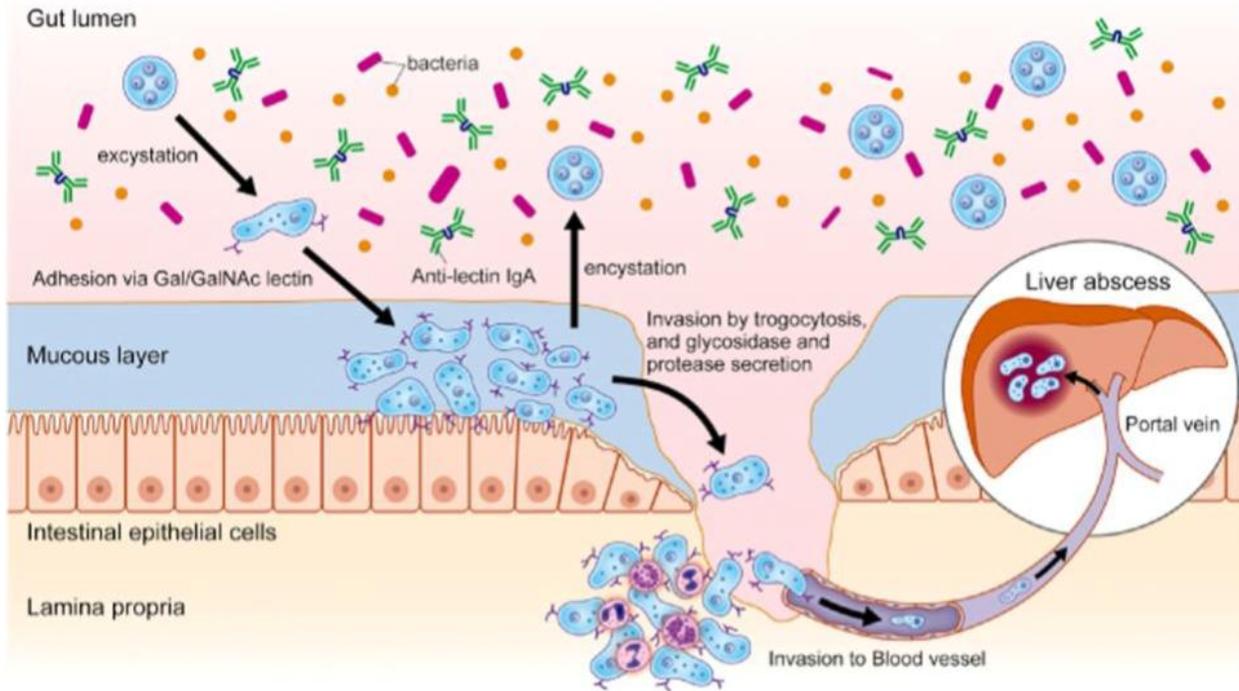
- ✓ producing sores (ulcers),
- ✓ bloody diarrhea,
- ✓ severe abdominal cramps,
- ✓ vomiting,
- ✓ chills, and fevers as high as 40°C.
- ✓ In addition, a case of acute amoebic dysentery may cause complications, including inflammation of the appendix, a tear in the intestinal wall (perforation), or a sudden, severe inflammation of the colon (fulminating colitis).



Extraintestinal Amoebiasis

### Hepatic Amoebiasis

It is the most common extraintestinal complication of amoebiasis. The center of the An abscess contains thick, chocolate-brown pus (anchovy sauce pus).



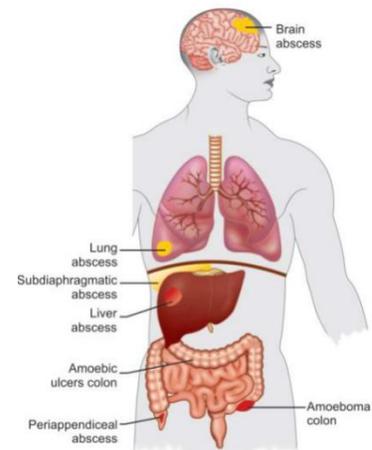
### Hepatic Amoebiasis

### Pulmonary Amoebiasis

Very rarely, lung amoebiasis may occur via direct hematogenous spread from the colon, which passes through the liver.

### Metastatic Amoebiasis

Involvement of distant **organs occurs via hematogenous spread and lymphatic drainage**. Abscesses in the **kidney, brain, spleen, and adrenals** have been noticed.





## Laboratory Diagnosis

### Stool examination

Microscopic Appearance: **Actively motile trophozoites, throwing pseudopodia**, can be demonstrated in freshly passed stool. Presence of **ingested RBCs clinches** the **identity** of *E. histolytica*. Cyst has a smooth, thin cell wall and contains round, refractile chromatoid bars and four nuclei.

### Serodiagnosis

Serological tests become positive only in invasive amoebiasis.

### Treatment

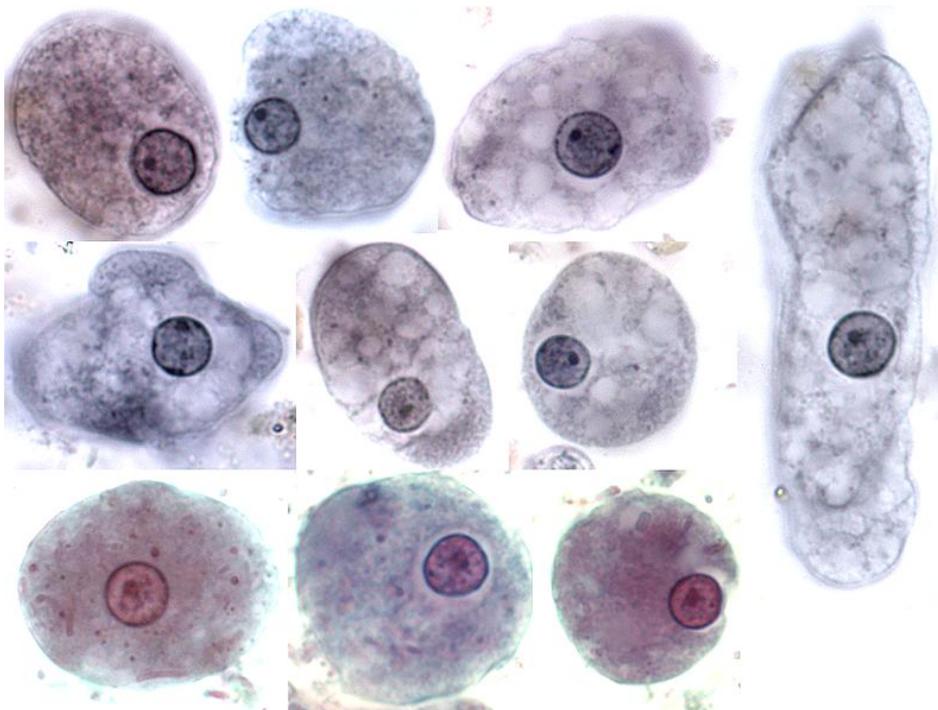
Three classes of drugs are used in the treatment of amoebiasis.

- Luminal amoebicides:
- Tissue amoebicides:
- Both luminal and tissue amoebicides:

## *Entamoeba Coli*

### Laboratory diagnosis of amebiasis is made by stool examination.

- It is widely distributed and a nonpathogenic commensal intestinal amoeba.
- It is larger than *E. histolytica*, about 20–50  $\mu\text{m}$ , with sluggish motility, and contains ingested bacteria but no red cells.
- The nucleus is clearly visible in unstained films and has a large eccentric karyosome and thick nuclear membrane lined with coarse granules of chromatin
- Cysts are large, measuring 10–30  $\mu\text{m}$ , with a prominent glycogen mass in the early stage. The chromatoid bodies are splinter-like and irregular.
- The mature cyst has 8 nuclei
- The life cycle is the same as in *E. histolytica* except that it remains a luminal commensal without tissue invasion and is nonpathogenic.





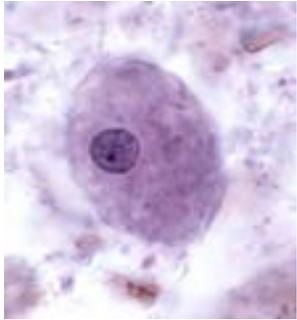
Characteristic	Troph. of <i>E. histolytica</i>	Troph. of <i>E. coli</i>
Size	8-65µm	12-55µm
No. of nuclei	One	one
Karyosome	Small & <b>central</b>	Large irregular shape, <b>eccentric</b>
Peripheral chromatin	Fine& evenly distributed	Coarse & unevenly distributed
Cytoplasm	Finely granular	Coarse& often vacuolated
Cytoplasmic inclusion	Ingested RBC	Bacteria, other debris
Motility	Progressive, finger like pseudopodia	Non Progressive, blunt pseudopodia
Figure		

Table showing the comparison between trophozoite of *E. Histolytica* & *E. coli*



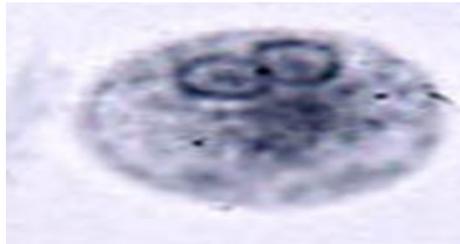
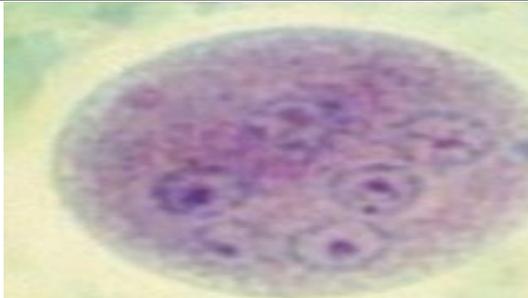
characteristic	cyst of <i>E. histolytica</i>	cyst of <i>E. coli</i>
Size	8-22µm	8-35µm
shape	Spherical to round	Spherical to round
No. of nuclei	One to four	One to eight
Karyosome	Small & central	Large irregular shape, eccentric
Peripheral chromatin	Fine & evenly distributed	Coarse
Cytoplasm	Finely granular	granular
Cytoplasmic inclusion	Chromatoid bars, rounded ends, diffuse glycogen mass	Chromatoid bars, rounded with pointed ends, diffuse glycogen mass
Figure		

Table showing the comparison between cysts of *E. Histolytica* & *E. coli*



## Key points of Amoebae

- **Definitive host:** The host in which the adult stage lives or the sexual mode of reproduction takes place.
- **Intermediate host:** The host in which the larval stage of the parasite lives or where asexual multiplication takes place.
- **Zoonoses:** Diseases which can be transmitted to humans from animals, e.g., malaria, leishmaniasis, trypanosomiasis, and echinococcosis.
- Some protozoa have a kinetoplast and flagella or cilia.
- Amoebae move by extending temporary protrusions of the body called pseudopodia.
- Trophozoites are the active feeding and growing stage of protozoa.
- Cysts are the resting or resistant stage of protozoa, bounded by a tough cell wall.
- *E. histolytica* is found in the human colon and is mainly asymptomatic.
- A cyst contains glycogen mass and 1–4 chromatid bars.
- **Amoebic ulcers:** Typical ulcers are discrete, flask-shaped, with a ragged, undermined margin, found in the caecum.
- **Extraintestinal complications:** Amoebic hepatitis and liver abscess are the most common.
- Abscesses in other organs such as the lung, brain, spleen, and genitourinary tract.

## *Balantidium Coli*

Largest protozoan parasite residing in the large intestine of man: *Balantidium coli*.  
It is the only **ciliate protozoan** parasite of humans.

### Morphology

*B. Coli* occurs in 2 stages – trophozoite and cyst

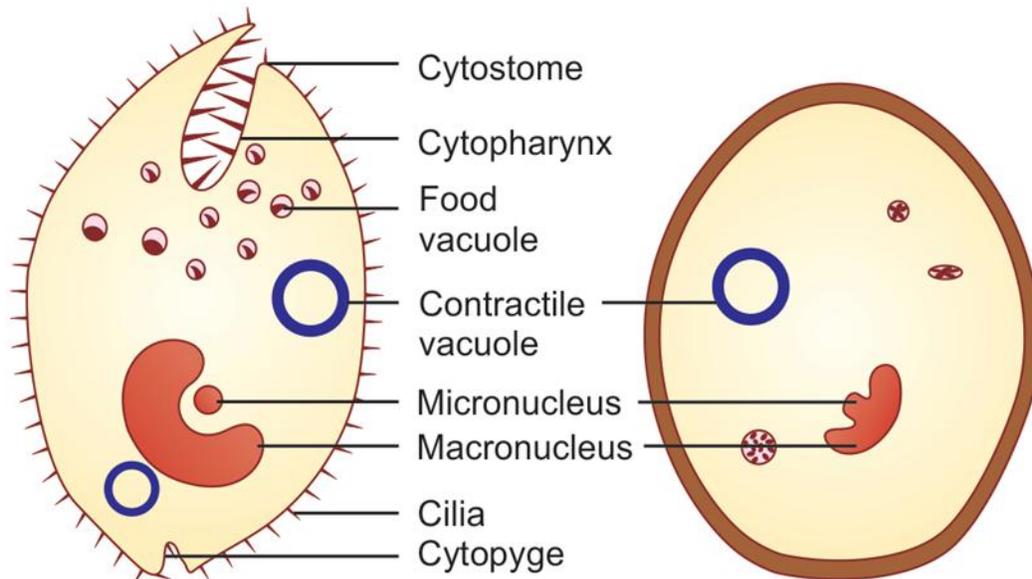


Fig.1. Morphology of *Balantidium coli*. **A.** Trophozoites; **B.** Cyst

#### 1- Trophozoite

The trophozoite lives in the large intestine, feeding on cell debris, bacteria, starch grains, and other particles. The trophozoite is actively motile and is invasive stage of the parasite found in dysenteric stool.

#### 2- Cyst

The cyst is the **infective stage** of *B. coli*. It is found in chronic cases and carriers.

## Life Cycle

*B. coli* passes its life cycle in one host only (monoxenous).

**Natural host:** Pig.

**Accidental host:** Man.

**Reservoirs:** Pig, monkey, and rat.

**Infective form:** Cyst.

- ✓ **Mode of transmission:** Balantidiasis is a zoonosis. Human beings acquire infection by ingestion of **food and water contaminated with feces containing the cysts of *B. coli***.
- ✓ Infection is acquired from pigs and other animal reservoirs or from human carriers.
- ✓ Once the cyst is ingested, **excystation occurs in the small intestine**
- ✓ From each cyst, a **single trophozoite is produced which migrates to large intestine**.
- ✓ Liberated trophozoites multiply in the large intestine by transverse binary fission.
- ✓ **Encystation occurs** as the trophozoite passes down the colon or in the evacuated stool. In this process, the cell rounds up and secretes a tough cyst wall around it.
- ✓ The cysts remain viable in feces for a day or 2 and may contaminate food and water, thus it is **transmitted to other human or animals**.

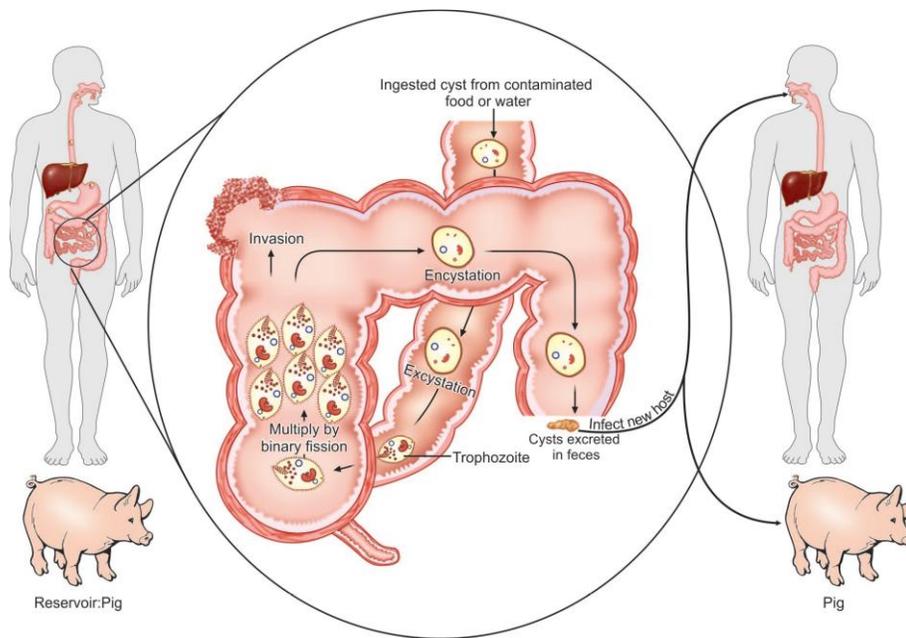


Fig 2; Life cycle of *Balantidium coli*



## Clinical Features

Most infections are asymptomatic.

- ✓ Symptomatic disease or **balantidiasis** resembles **amoebiasis** causing **diarrhea** or frank **dysentery** with abdominal colic, tenesmus, nausea, and vomiting.
- ✓ *Balantidium* **ulcers may be secondarily infected by bacteria.**
- ✓ In chronic balantidiasis, patients have **diarrhea alternating with constipation.**

### Laboratory Diagnosis:

#### Stool Examination

Diagnosis of *B. coli* infection is established by demonstration of **trophozoites and cysts** in feces.

### Treatment:

- ✓ **Tetracycline** is the drug of choice
- ✓ Alternatively Doxycycline can be give.
- ✓ Metronidazole and nitroimidazote have also been reported to be useful in some cases.

### Prophylaxis

- ✓ Avoidance of contamination of food and water with human or animal feces.
- ✓ Prevention of human-pig contact.
- ✓ Treatment of infected pigs.
- ✓ Treatment of individuals shedding *B. coli* cysts.

## Intestinal Flagellates/ Giardia lamblia

### Habitat

*G. lamblia* lives in the **duodenum and upper jejunum** and is the only protozoan parasite found in the **lumen of the human small intestine.** m)

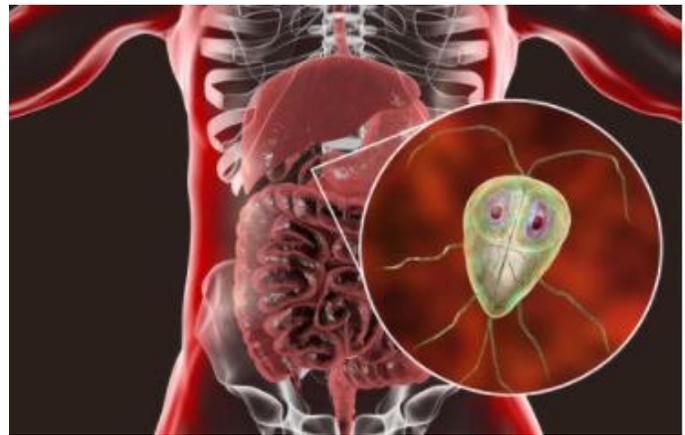


Fig 3. Giardia lamblia in the human duodenum, computer illustration.

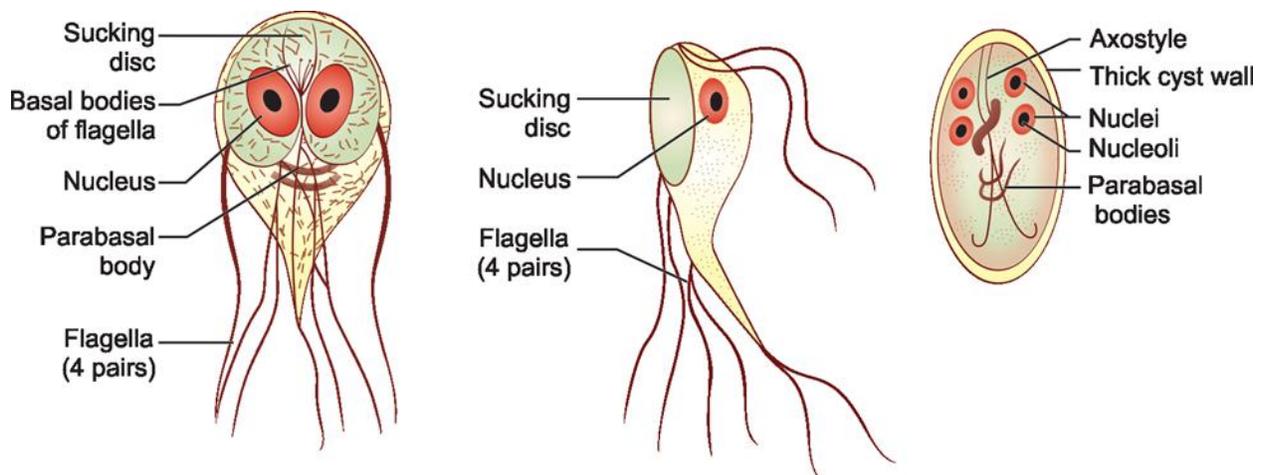


Fig. 4; Trophozoite. **A.** Ventral view; **B.** Lateral view; **C.** Quadrinucleate Cyst

### Trophozoite

It is bilaterally symmetrical and possesses.

- ✓ 1 pair of nuclei
- ✓ 4 pairs of flagella
- ✓ Blepharoplast, from which the fl agella arise (4 pairs)
- ✓ 1 pair of axostyles, running along the midline

The trophozoite is motile, with a slow oscillation about its long axis, often resembling **falling leaf**.

## Cyst

It is the infective form of the parasite

## Life Cycle

Giardia passes its life cycle in **1 host**.

**Infective form:** Mature cyst.

**Mode of transmission:**

- ✓ Man acquires infection by ingestion of **cysts in contaminated water and food**.
- ✓ Within half an hour of ingestion, the cyst **hatches out into two trophozoites**, which multiply successively by **binary fission and colonize in the duodenum**.
- ✓ The trophozoites live in the **duodenum and upper part of jejunum**, feeding by pinocytosis.
- ✓ During unfavorable conditions, **encystment occurs usually in colon**.
- ✓ Cysts are passed in stool and remain viable in soil and water for several weeks.
- ✓ There may be 200,000 cysts passed per gram of feces.
- ✓ **Infective dose** is 10–100 cysts

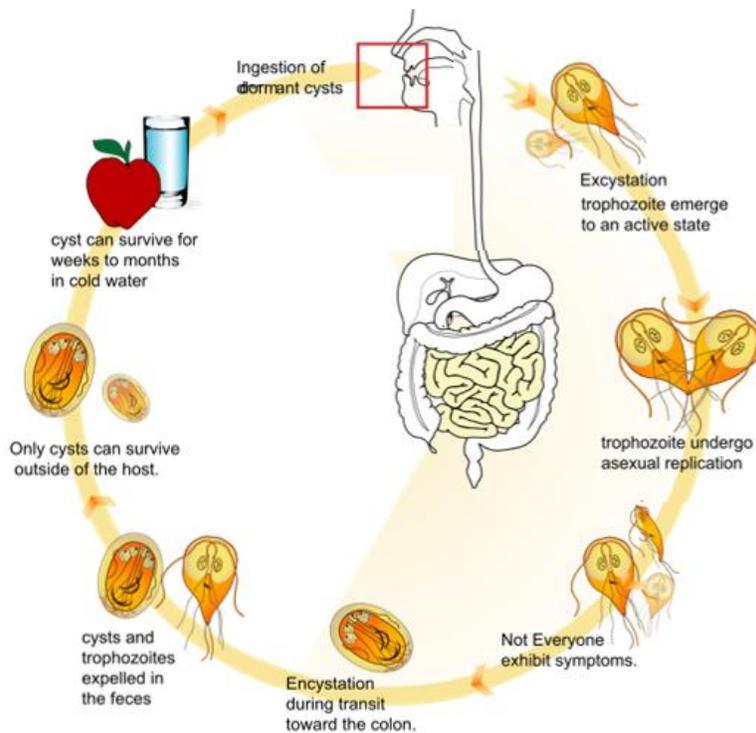


Fig-4 *Giardia lamblia* life cycle

## Pathogenicity and Clinical Features

- ✓ *G. lamblia* **does not invade the tissue**, but remains tightly **adhered to intestinal** epithelium by means of the **sucking disc**.
- ✓ They may cause abnormalities of **villous architecture** by cell apoptosis and increased lymphatic infiltration of lamina propria.

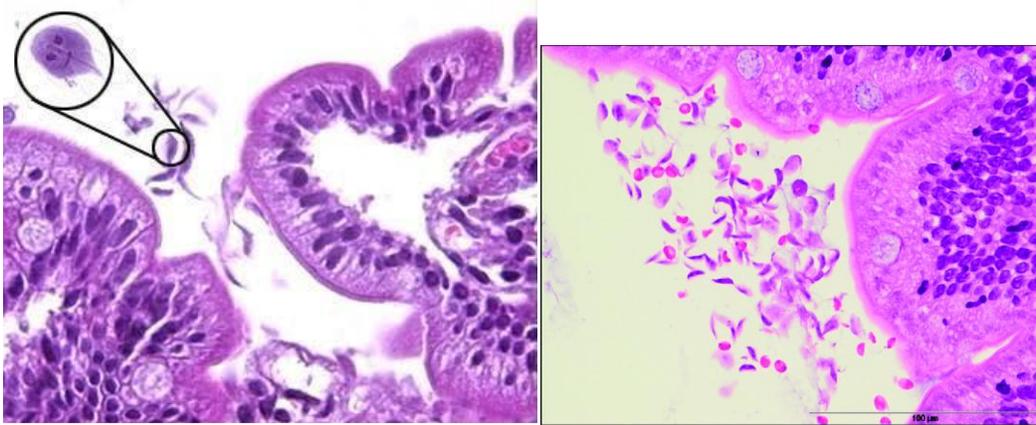


Fig. 5 Giardia lamblia in duodenal biopsy specimens

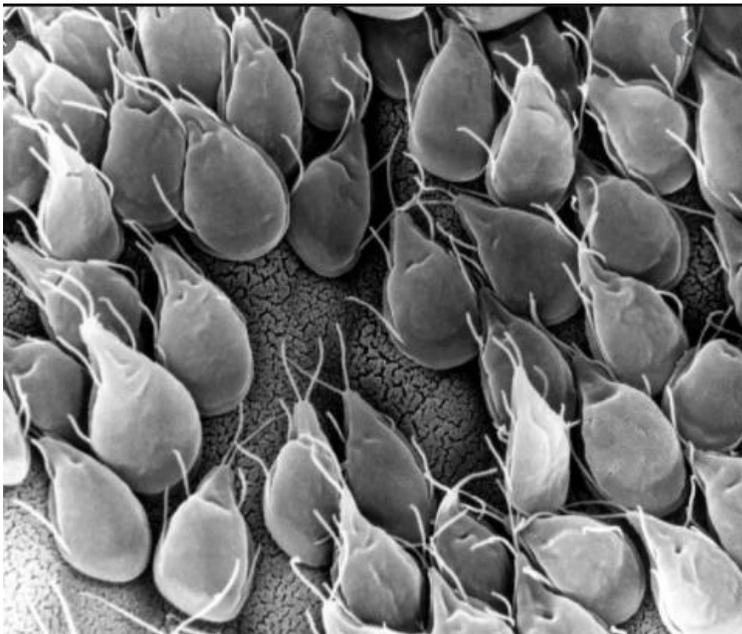


Fig 6. This scanning electron micrograph (SEM) shows the mucosal surface of the small intestine of a gerbil infested with Giardia



## Clinical Features

- ✓ Often they are asymptomatic, but in some cases, *Giardia* may lead to **mucus diarrhea, fat malabsorption** (steatorrhea), dull **epigastric pain**, and flatulence. The stool contains excess **mucus and fat but no blood**.
- ✓ Children may develop **chronic diarrhea, malabsorption of fat, vitamin A, protein, sugars like xylose disaccharides**, and weight loss.
- ✓ Occasionally, *Giardia* may colonize the **gall bladder**, causing **biliary colic and jaundice**.
- ✓ Incubation period is variable, but is usually about **2 weeks**.

## Laboratory Diagnosis

### Stool Examination

- ✓ Giardiasis can be diagnosed by identification of **cysts of *Giardia lamblia* in the formed stools** and the **trophozoites and cysts of the parasite in diarrheal stools**.
- ✓ On macroscopic examination fecal specimens containing *G. lamblia* may have an **offensive odor**, are **pale colored and fatty**, and **float in water**.
- ✓ On microscopic examination, **cysts and trophozoites** can be found in diarrheal stools by **saline and iodine wet preparations**.

## Treatment

1. Metronidazole.
2. Tinidazole is more effective than metronidazole.
3. Furuzolidone and nitazoxamide are preferred **in children**
4. Parmomycin, an oral aminoglycoside can be given to **symptomatic pregnant females**.

## Prophylaxis

Giardiasis can be prevented by following measures:

- ✓ Proper disposal of **waste water and feces**.
- ✓ Practice of **personal hygiene like hand-washing** before eating and proper disposal of diapers.
- ✓ Prevention of food and water contamination. **Community chlorination** of water is **ineffective for inactivating cysts**. **Boiling** of water and filtration by membrane filters are required.