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Medical Microbiology II (2022-2023)



Flagellates Protoza

Flagellates are protozoa that bear one to several long, delicate, thread-like extensions of the cytoplasm. These are known as flagella (singular, flagellum). According to their habitat, the flagellates are classified into two broad groups (Table 4.1, Flagellates P.42, Arora).

1. Intestinal, oral and genital flagellates
2. Blood and tissue flagellates.

Intestinal, Oral and Genital Flagellates

Giardia lamblia (G.duodenalis, G.intestinalis)

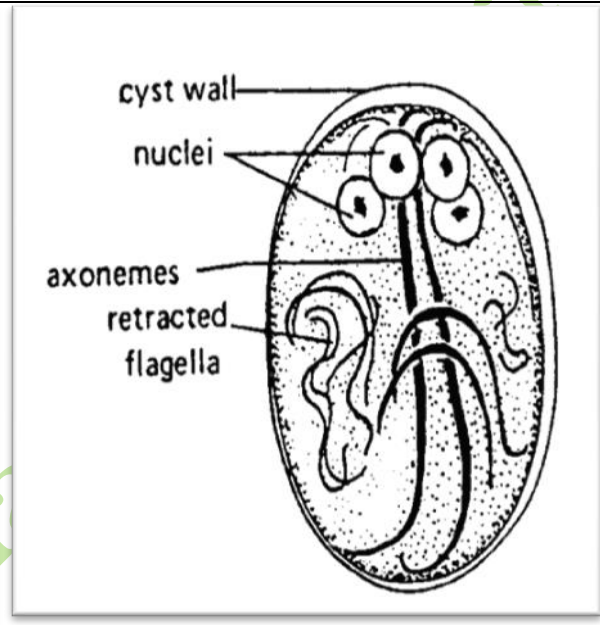
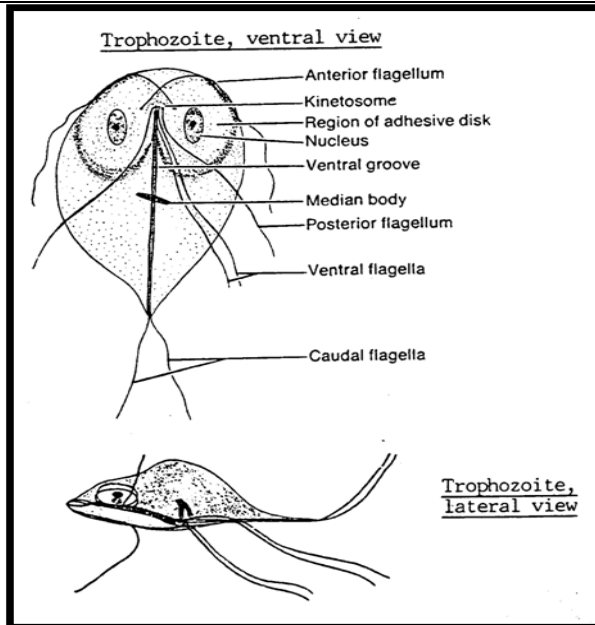
Geographical distribution *Giardia lamblia* is a cosmopolitan parasite. The highest prevalence of *G. lamblia* occurs in tropics and sub tropics where sanitation is poor. Infections seem to be more common in children than adults. *Giardia lamblia* causes giardiasis.

Habitat It inhabits duodenum and the upper part of jejunum of man.

Morphology It exists in two forms: 1. Trophozoite 2. Cyst.

| trophozoite | cyst |
|---|--|
| <p>Trophozoite is pear-shaped with rounded anterior and pointed posterior. It measures 10–20 μm in length and 5–15 μm in width.</p> <p>The dorsal surface is convex while on the ventral surface it has a shallow posteriorly notched concavity (sucking disc) that embraces anterior half of the organism. It acts as an organelle of attachment.</p> <p>It is bilaterally symmetrical and has</p> | <p>Mature cyst is oval in shape and measures 11–14 μm \times 7–10 μm in size. It has two pairs of nuclei which may remain clustered at one end or lie in pairs at opposite poles. The remains of the flagella and margins of the sucking disc may be seen inside the cytoplasm of the cyst.</p> |

one pair of nuclei, four pairs of flagella. The nuclei are rounded and possess a central karyosome. By rapid movement of the flagella, the trophozoites move from place to place, and by applying their sucking discs to epithelial surfaces they become firmly attached.



Culture

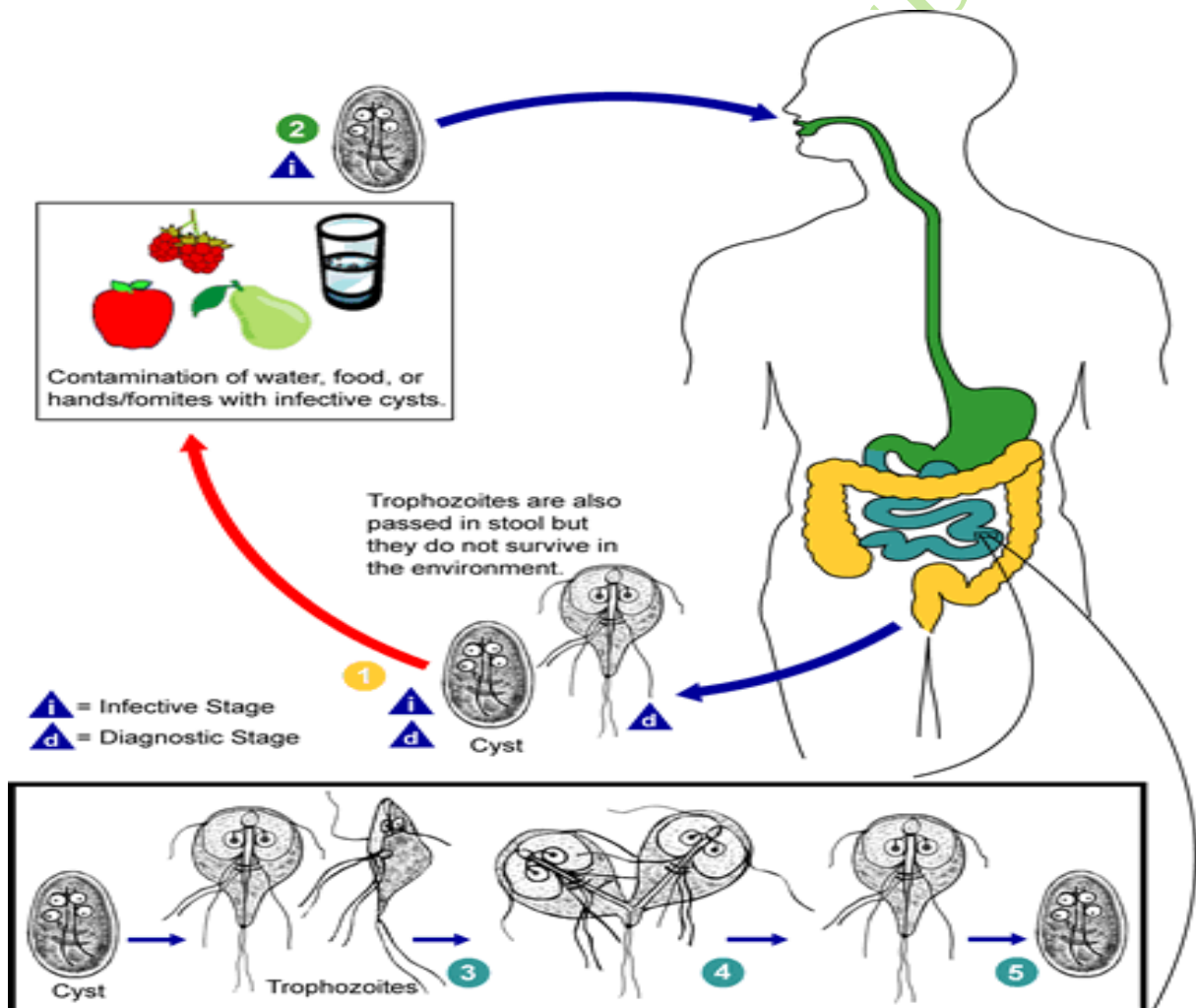
G. lamblia can be grown axenically in **Diamond's medium**.

Life cycle

- **It passes** its life cycle in a single host, the man. No intermediate host is required.
- **Mature cyst** is the infective form of the parasite. Man acquires infection by ingestion of cysts in faecally contaminated water or food. Infection may occur through ingestion of as few as 10–25 cysts. Infection may also be acquired by anal-oral sexual practices among male homosexuals.
- **Within 30** minutes of ingestion excystation occurs in the duodenum. The cyst hatches out two trophozoites, which then multiply by binary fission

to form enormous numbers and colonize in the duodenum and upper part of jejunum.

- **To avoid** acidity of duodenum, it may localize in biliary tract. the trophozoite attaches to the mucosal surface of the duodenum and jejunum. In frankly diarrhoeic stools, it is usual to find only the trophozoites. Encystation occurs commonly in transit down the colon where the intestinal contents lose moisture and patient starts passing formed stools. The trophozoites retract the flagella into the axonemes, the cytoplasm becomes condensed and a thin tough hyaline cyst wall is secreted. As the cyst matures, the internal structures are doubled, so that when excystation occurs, the cytoplasm divides, thus producing two trophozoites.



Life cycle of *G.lamblia*

Antigenic variation

G.lamblia is known to undergo surface antigenic variation. The antigens involved belong to a group of variant-specific surface proteins that are unique cysteine-rich zinc finger proteins. This may provide a mechanism enabling the organism to escape the host's immune response.

Pathogenicity

- **G. Lamblia** flagellates do not invade the tissues, but feed on mucous secretions.
- **With** the help of sucking disc the parasite attaches itself to the surface of the epithelial cells in the duodenum and jejunum, and in an appreciable number of cases it may cause duodenal and jejunal irritation leading to duodenitis and jejunitis.
- **The incubation** period, ranges from 1–4 weeks (average, 10 days).
- **Patient** may complain of dull epigastric pain, flatulence and chronic diarrhoea of steatorrhoea type.
- **The stool** is voluminous, foul smelling and contains large amount of mucus and fat but no blood. This is due to **malabsorption** since the parasites are coated on the mucosa, thus absorption suffers. Patient loses weight.
- **When** the parasite localizes in the biliary tract, it may lead to chronic cholecystitis and jaundice.
- **Giardiasis** is one of the more common causes of traveller's diarrhoea. Visitors to areas endemic for Giardia are more likely to present with symptoms than individuals who live in the area. This difference is probably due to the development of immunity from prior, and possibly continued, exposure to the organism.

Immune response Studies have confirmed the presence of both humoral and cellular immune responses to Giardia. The majority of infected patients produce detectable levels of Giardia-specific antibodies.

Laboratory diagnosis

Microscopy

Giardiasis can be diagnosed by identification of cysts of *G. lamblia* in the formed stools and the trophozoites of the parasite in diarrhoeal stools by normal saline and iodine preparation, and iron haematoxylin stain

a series of even five or six consecutive stool specimens may not show any parasite.

Trophozoites of *G. lamblia* may be detected in the bile aspirated from duodenum by intubation and by duodenal capsule technique (Enterotest) Motility of the trophozoites on wet preparations may be difficult to see because the organisms are caught up in mucus.

For the detection of *G. lamblia* in faecal specimens, a fluorescent method using monoclonal antibodies is extremely sensitive and specific

Biops

After multiple stool examinations, examination of bile aspirated from duodenum and Enterotest are negative, biopsy from multiple duodenal and jejunal sites may confirm the diagnosis of giardiasis. Touch preparations can be air dried, fixed in methanol, and stained with Giemsa stain. Trophozoites may appear purple and epithelial cells appear pink.

Immunologic tests

- ELISA test has been developed for the detection of Giardia antigen in faeces.
- Anti-Giardia antibodies, in the patient serum, may be detected by ELISA and indirect fluorescent antibody tests.

Treatment

Chemotherapy with a 5-day course of **metronidazole** usually is successful, although therapy may have to be repeated or prolonged in some instances.

A single dose of **tinidazole** probably is superior to metronidazole for treatment of giardiasis.

Paromomycin has been used to treat pregnant women to avoid any possible mutagenic effects of other drugs.

Prevention Giardiasis can be prevented by:

- improved water supply,
- proper disposal of human faeces,
- improved personal hygiene,
- routine hand-washing,
- proper storage of food and water,
- control of insects which may come in contact with infected stools and then contaminate food or water, and
- treatment of symptomatic and asymptomatic individuals. Prospects are poor for the development of a potential vaccine

Chilomastix mesnili

Chilomastix mesnili is a common flagellate living as a harmless commensal in the caecum and colon of man. It has a cosmopolitan distribution but is more prevalent in warm than in cool climates. It has well-defined trophozoite and cystic stages.

Morphology Trophozoite It is pear-shaped measuring 6–20 μm in length and 3–10 μm in breadth. The posterior end of the trophozoite is drawn out into a long cone. The spherical nucleus, measuring 3–4 μm in diameter is situated anteriorly. It has a small distinct, central karyosome and a few achromatic fibrils extending to the nuclear membrane, and chromatin plaques lining the membrane. A large conspicuous cytostome (mouth) is seen on one side of the nucleus. It has three free anterior flagella and a delicate flagellum lying within the cytostome.

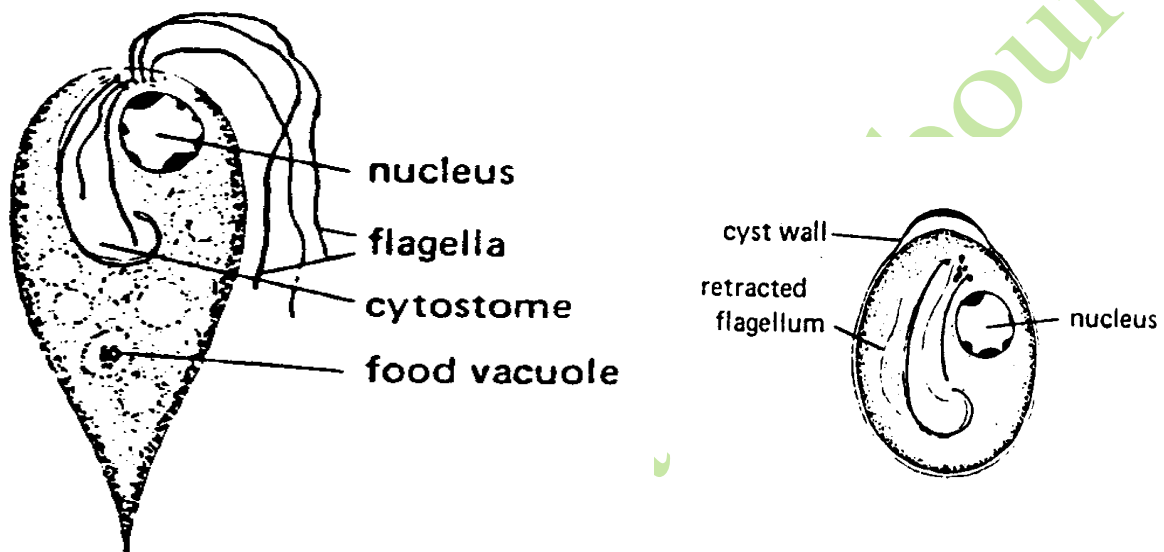
Cyst It is lemon-shaped with a small projection at the anterior end. It measures 7–10 μm in length and 4–6 μm in breadth and is surrounded by a thick tough cyst wall. The single nucleus lies near the centre.

Habitat *C. mesnili* is a normal inhabitant of the caecum and colon of man. In freshly passed liquid stools, only trophozoites are seen, in semi-formed stools, both trophozoites and cysts may be observed, and in well-formed stools, only cysts are present.

Transmission of the parasite, from one person to another, takes place by ingestion of food or water contaminated with cysts of *C. mesnili* in the stools of an infected individual.

Pathogenicity and laboratory diagnosis *C. mesnili* is a harmless commensal and does not produce any symptom.

The diagnosis can be made by detection of trophozoites and cysts of *C. mesnili* in the faecal smear.



Trichomonas

Genus *Trichomonas* contains three species which occur in humans:

1. *T. tenax*
2. *T. hominis*
3. *T. vaginalis*.

These flagellates exist only in trophozoite stage. Cystic stage is absent. They have four anterior flagella and one lateral flagellum which is attached to the surface of the parasite to form undulating membrane. The undulating membrane is supported at the base by a rod-like structure known as costa.

Trichomonas tenax

It is a pyriform flagellate. It measures 5–12 μm in length and 5–10 μm in width. It is a harmless commensal of the human mouth, living in the tartar around the teeth, in cavities of carious teeth, in necrotic mucosal cells in the gingival margins of gums and in pus pockets in tonsillar follicles. It is transmitted by kissing, salivary droplets and fomites. Although *T. tenax* is considered to be harmless commensal in the mouth, there are reports of respiratory infections and thoracic abscesses.

Diagnosis can be made by demonstration of *T. tenax* in the tartar by microscopy, and no therapy is indicated. Better oral hygiene will rapidly eliminate the infection.

Trichomonas hominis

It is pyriform, measuring 5–14 μm in length and 7–10 μm in width. It inhabits the caecum of man and several other primate species and feeds on enteric bacteria. It does not invade the intestinal mucosa. Though it has occasionally been found in the diarrhoeic stools, its pathogenicity is yet to be established. In freshly passed specimens, particularly in unformed stools, the motility may be visible. In wet preparation, look for the flagellar movement, undulating membrane and the presence of the axostyle

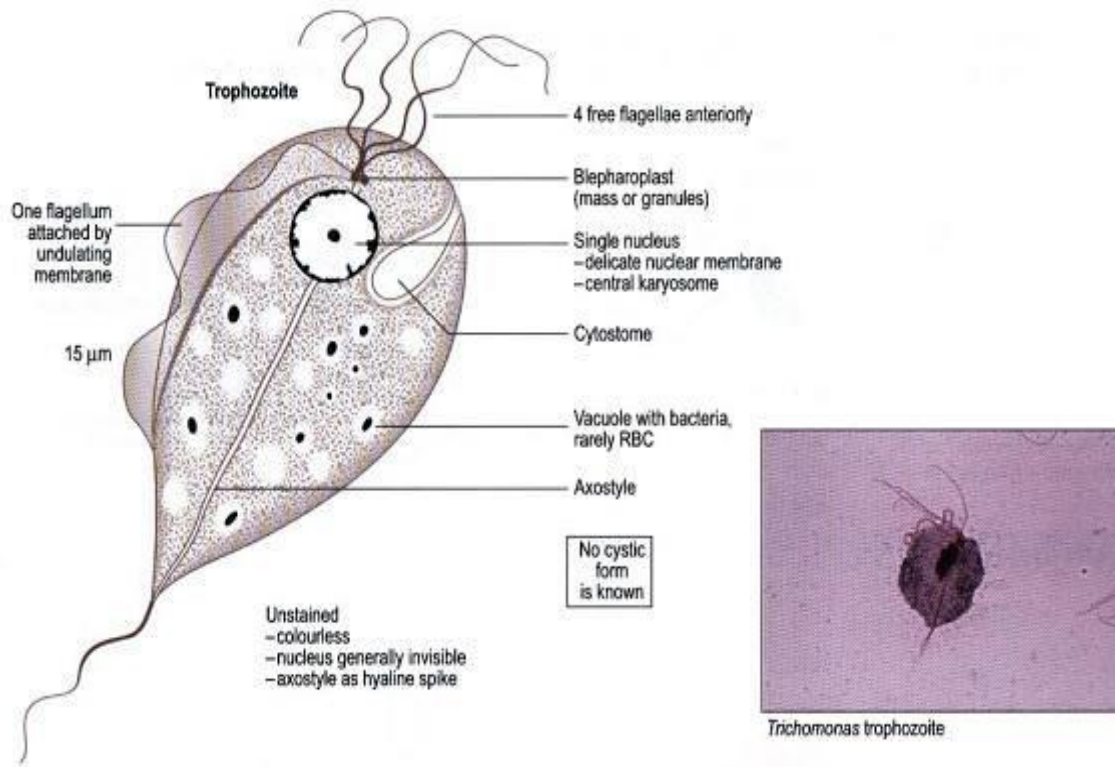
Trichomonas vaginalis

Trichomonas vaginalis was first observed by Donne in 1836. It has worldwide distribution with higher prevalence among persons with multiple sexual partners or other venereal diseases.

Morphology It resembles *T. tenax* but it is larger than this. It measures 7–23 μm in length and 5–15 μm in width. In a wet mount under the low-power objective the trophozoites show jerky movement. High power examination may reveal the beating flagella and undulating membrane characteristic of the species.

Habitat

The normal habitat of the parasite is the vagina and urethra of women, and the urethra, seminal vesicles and prostate of man. It may also be found in the Bartholin's glands and in urinary bladder in females.



Pathogenicity

The parasite lives on the mucosa feeding on bacteria and leucocytes. *T. vaginalis* is an obligate parasite.

Asymptomatic infections have been observed in 50% of infected female patients. The organism is responsible for a mild vaginitis with discharge. Vaginal discharge contains a large number of parasites and leucocytes and is liquid, greenish or yellow.

Male patients usually have mild or asymptomatic infections. They may develop itching and discomfort inside penile urethra, especially during urination. The parasite is transmitted by sexual intercourse.

The main mechanisms postulated seem to be mediated by cell to cell adhesion, haemolysis, excretion of soluble proteinases, pore-forming proteins and cell detaching factor.

Laboratory diagnosis

Microscopy

The diagnosis can be made by demonstration of trophozoites of *T. vaginalis* in wet mounts of the sedimented urine, vaginal secretions or vaginal scrapings by bright field, dark-field, or phase-contrast microscopy.

In males it may be found in urine or prostatic secretions. Fixed smears may be stained with Papanicolaou, Giemsa, Leishman and periodic acid-Schiff stain and seen under light microscope. The parasites may also be detected by fluorescence microscopy.

Culture

T. vaginalis can be isolated from urethral and vaginal exudates on several commercially available media. Trussell and Johnson's medium

It grows best at 35°–37°C under anaerobic conditions and less well aerobically. The optimal pH for growth is 5.5–6.0. Culture is very sensitive (95%) procedure for diagnosis of trichomoniasis. It is recommended when direct smear is negative.

Antibody and antigen detection Several types of ELISA have been developed for *T. Vaginalis*.

Molecular methods

- Nucleic acid hybridization methods for detection of *T. vaginalis* have sensitivity and specificity as good as culture methods.
- Polymerase chain reaction (PCR) for the diagnosis of trichomoniasis has also been developed.

Prevention Since infection is contracted through sexual inter course, therefore, the preventive measures include:

- detection and treatment of cases, both males and females;
- avoidance of sexual contact with infected persons.

There is no vaccine currently available for use against *T. vaginalis*.

Treatment Metronidazole, 2 g oral single dose or tinidazole, 2 g oral single dose are highly effective against *T. vaginalis* infection.

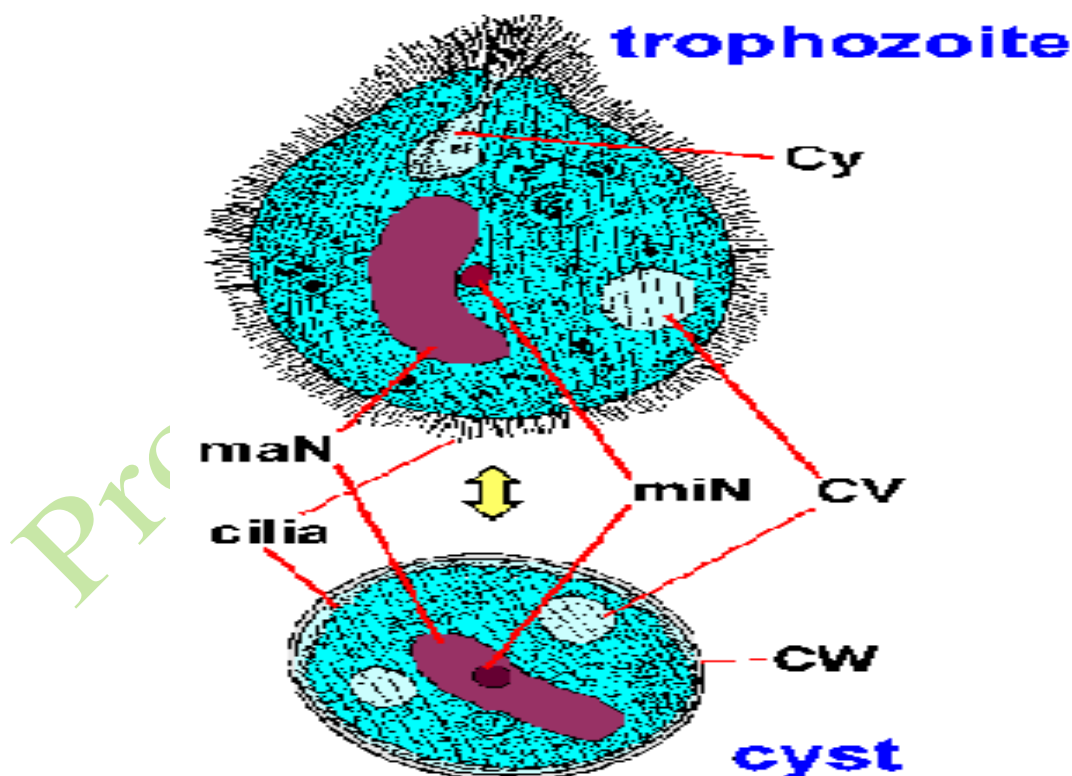
-Ciliates Protoza:

Balantidium coli

Balantidium coli, the cause of **balantidiasis** or balantidial dysentery, is the largest intestinal protozoan of humans.

Morphology & Identification:

The trophozoite is a ciliated, oval organism. The cell wall is lined with spiral rows of cilia. The cytoplasm surrounds two **contractile vacuoles**, food particles and vacuoles, and two nuclei—a large, kidney-shaped **macronucleus** and a much smaller, spherical genetic **micronucleus**. The macronucleus, contractile vacuoles, and portions of the ciliated cell wall may be visible in the cyst.



Pathogenesis, Pathology, & Clinical Findings:

-When cysts are ingested by the new host, the cyst walls dissolve and the released trophozoites descend to the **colon**, and form cysts that pass in the feces.

-However, rarely, the trophozoites invade the mucosa and submucosa of the large bowel and terminal **ileum**. As they multiply, abscesses and irregular ulcerations are formed.

-Chronic recurrent diarrhea, is the most common clinical manifestation, but there may be bloody mucoid stools, tenesmus, and colic.

Diagnostic Laboratory Tests:

-laboratory detection of trophozoites in liquid stools or cysts in formed stools.

-Sigmoidoscopy may be useful for obtaining material directly from ulcerations for examination.

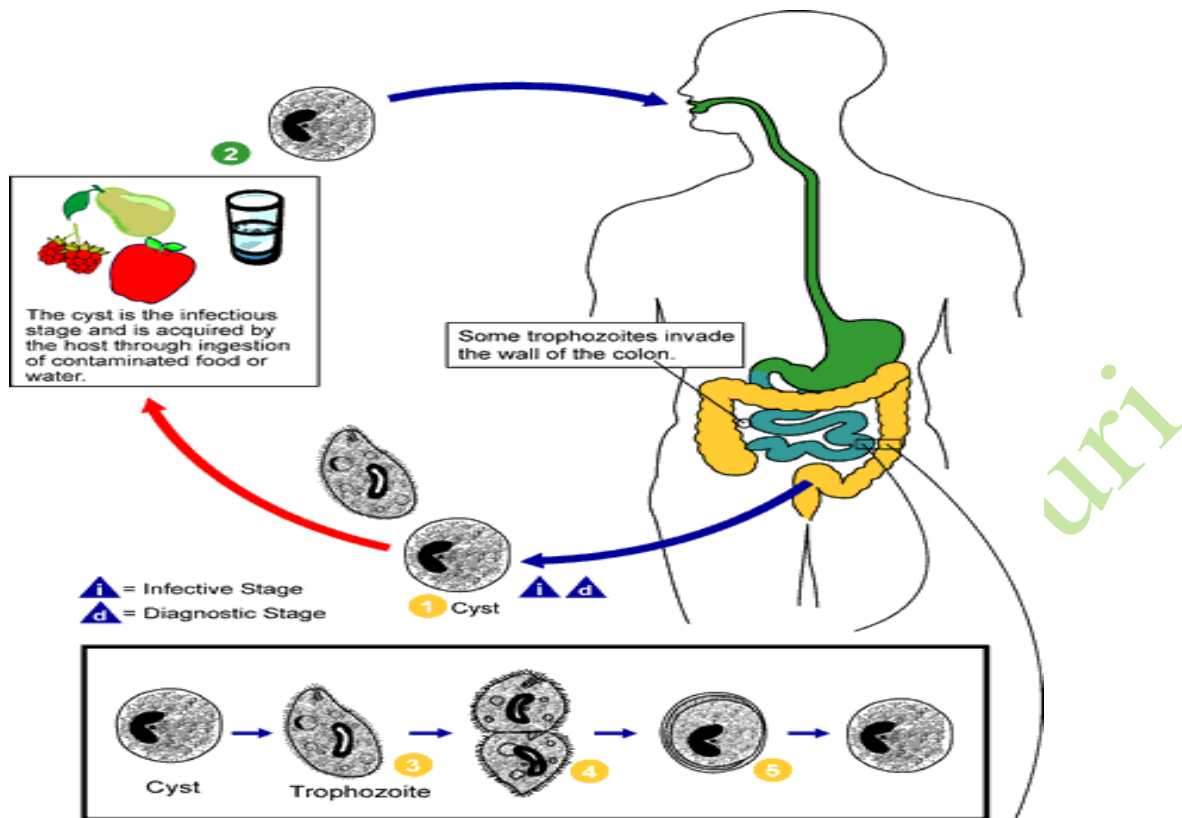
Treatment:

A course of **oxytetracycline** may be followed by **iodoquinol** or **metronidazole** if necessary.

Epidemiology:

-*B coli* is found in humans throughout the world, particularly in the tropics.

- Pig farmers and slaughterhouse workers are particularly at risk, though poor sanitation and crowding in jails, mental institutions are associated with infection.



Balantidium coli Life Cycle

References:

- Medical microbiology by Jawetz, 2007. Chapter 46: medical Parasitology
- Medical parasitology by Arora, 2018 (P 41-49).

Quiz/ What is the name of this parasite?

Answer: *Giardia lamblia*