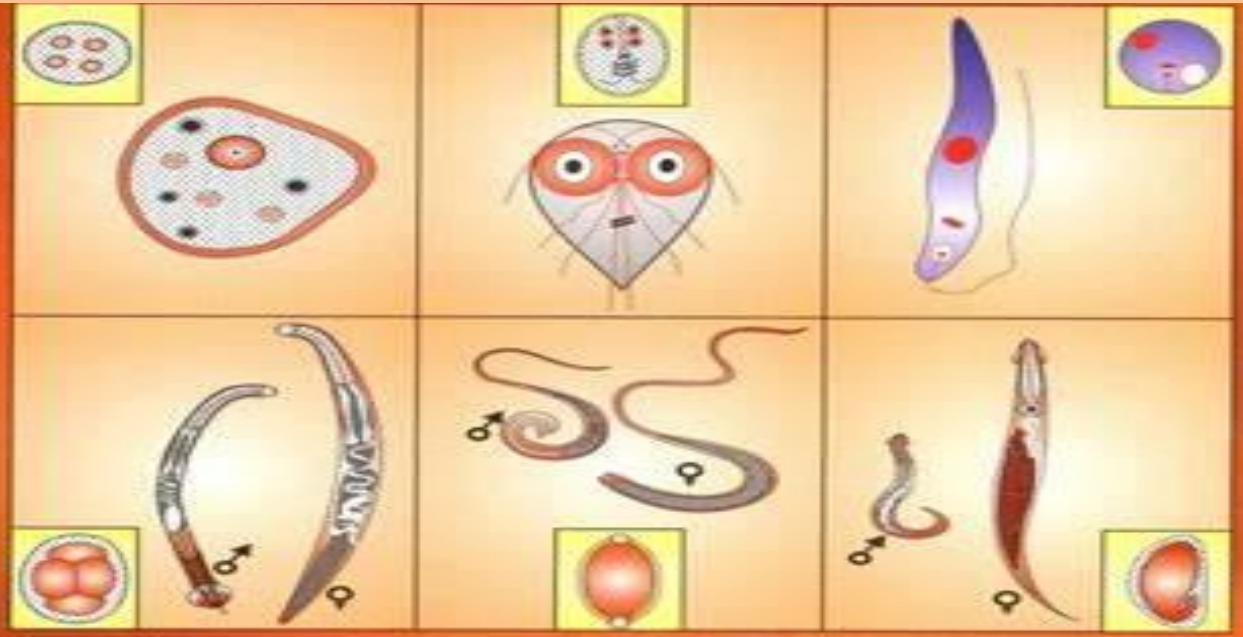
Tikrit University
College of Pharmacy

Microbiology II
-Medical Parasitology

MEDICAL PARASITOLOGY

Lec. 4: Intestinal, oral and genital flagellates



Assist. Prof. Dr. Maysam Adnan Mezher

A. Intestinal, oral and genital flagellates

1. *Giardia lamblia*

Giardia was discovered by Leeuwenhoek in 1681 in his own stool but was not described until 1859 by Lambl. The organism was named after Professor A. Giard of Paris and Professor F. Lambl of Prague.

Geographical distribution

Giardia lamblia is a cosmopolitan parasite. The highest prevalence of *G. lamblia* occurs in tropics and sub- tropics where sanitation is poor. Travellers to tropical Africa, Mexico, Russia, Southeast Asia, and western South America are at a high risk of acquiring giardiasis. *Giardia* infects 200 million people worldwide and may produce symptoms in 500,000 individuals every year. Infections seem to be more common in children than adults.

Habitat

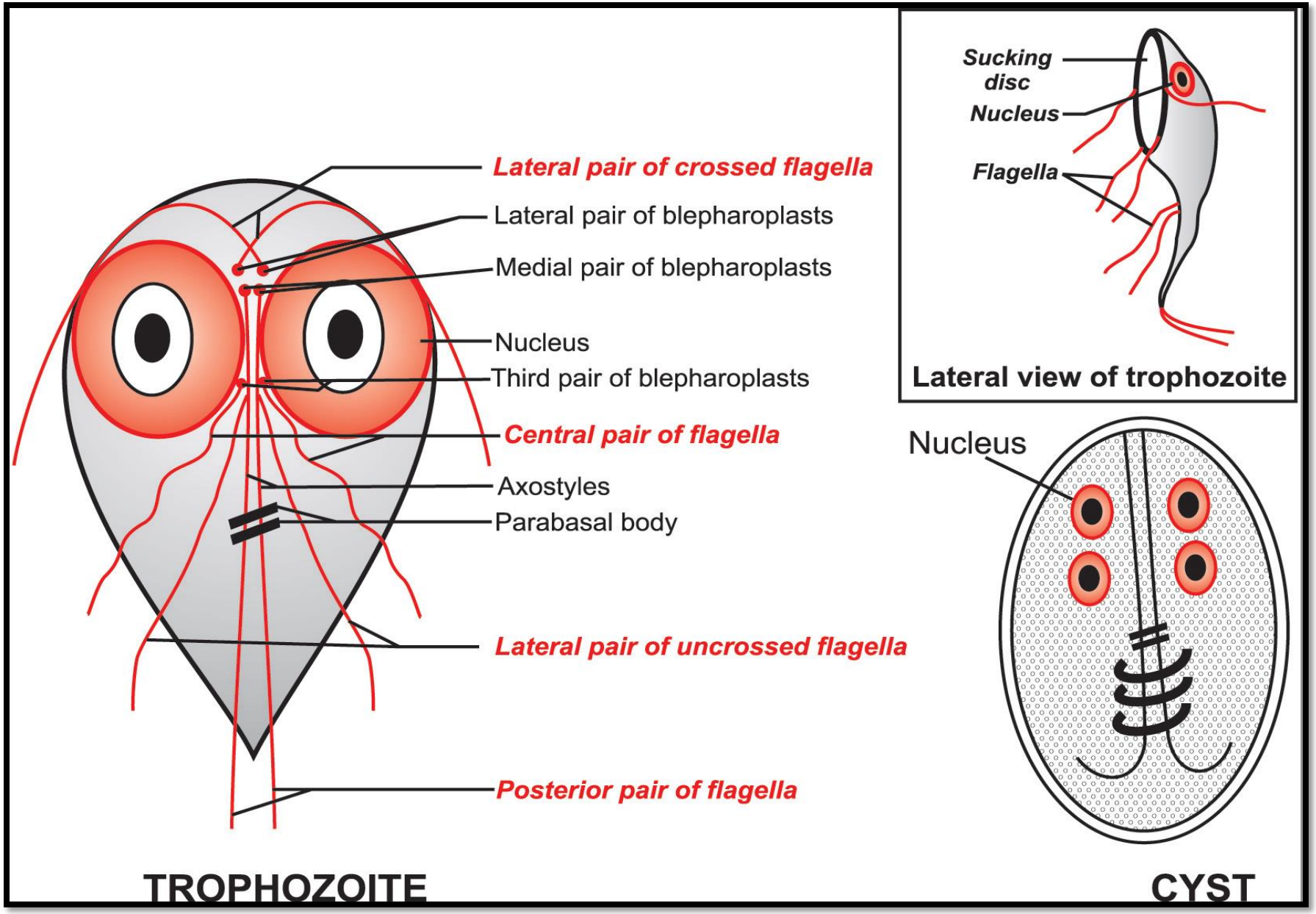
It inhabits duodenum and the upper part of jejunum of man.

Morphology

It exists in two forms: Trophozoite and Cyst

Trophozoite

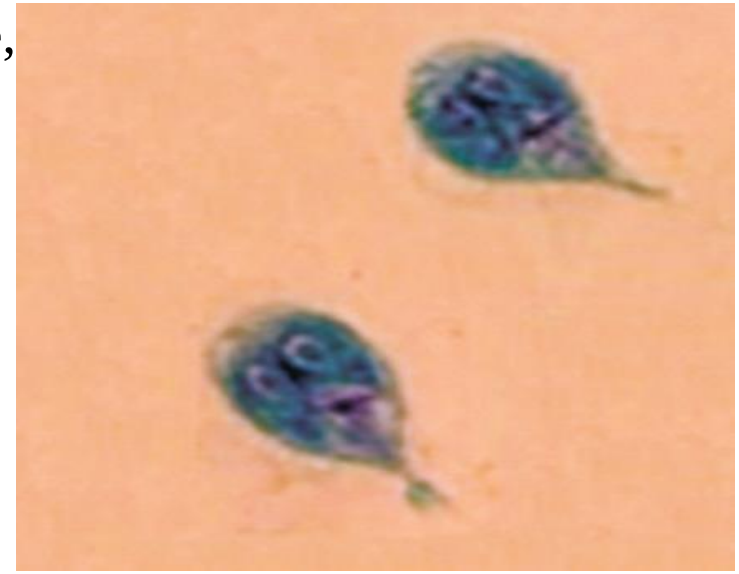
It is pear-shaped with rounded anterior and pointed posterior end. It measures 10–20 μm in length and 5–15 μm in width. The dorsal surface is convex while on the ventral surface it has a shallow posteriorly notched concavity (sucking disc) that embraces anterior half of the organism. It acts as an organelle of attachment. It is bilaterally symmetrical and has **one pair of nuclei**, one on each side of the midline, one pair of axostyles, one pair of parabasal bodies present on the axostyles, four pairs of flagella and probably four pairs of blepharoplasts from which the flagella arise.



Morphological forms of Giardia lamblia

• Two pairs of blepharoplasts (one lateral and one more median) are situated on each side of the midline, between and slightly anterior to the two nuclei. Two axonemes (also known as axostyles) arise from the median pair of blepharoplasts. These axonemes pass out from the posterior end of the body and give rise to posterior (caudal) pair of flagella.

• From the two lateral blepharoplasts there originate two axonemes that proceed forward in a curved course towards the midline, cross each other, then describe a wide arc and give rise to lateral pair of crossed flagella.



. Trophozoite of Giardia lamblia in stool (iron haematoxylinstain, × 400).

• Third pair of blepharoplasts lies near the centre of the sucking disc. They give rise to short axonemes and central pair of flagella.

• Fourth pair of blepharoplasts has not been located, but the axonemes that arise from them have been traced up to the notch of the sucking disc. This pair of blepharoplasts gives rise to lateral pair of uncrossed flagella. The trophozoites are motile by rapid movement of the flagella.

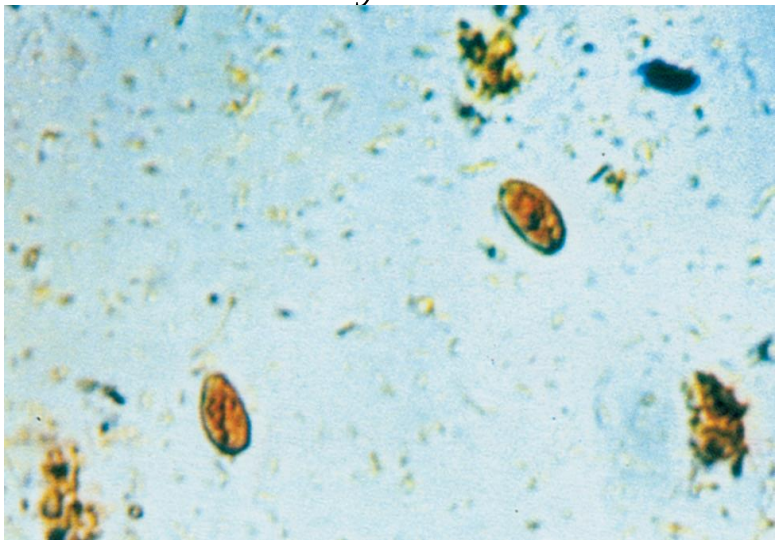
The **nuclei** are rounded and possess a central karyosome. The nuclear membrane is delicate and is not lined by chromatin material. By rapid movement of the flagella, the trophozoites move from place to place, and by applying their sucking discs to epithelial surfaces they become firmly attached.

Cyst

Mature cyst is oval in shape and measures 11–14 μm × 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 .

Culture

G. lamblia can be grown axenically in Diamond's medium, the medium also used for axenic cultivation of *E. histolytica*.



Cysts of Giardia lamblia in stool (iodine preparation, × 400).

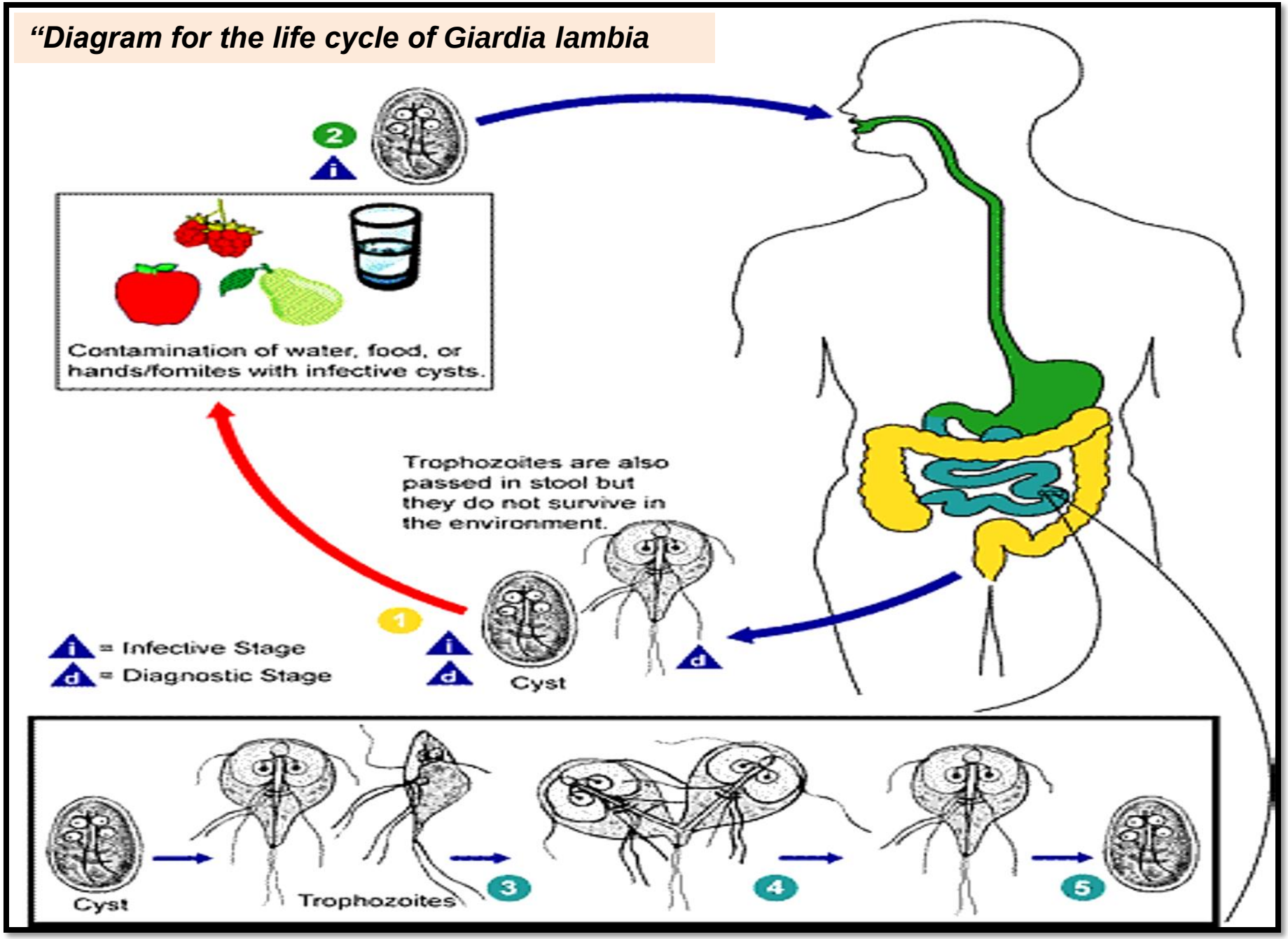
Life cycle

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.

By means of the concavity on its ventral surface 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.

“Diagram for the life cycle of Giardia lambia



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

The presence of *G. lamblia* in the glandular crypts of duodenal-jejunal mucosa may not cause any pathology. These 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, before symptomatic disease develops, 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 malabsorptionsince 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. Although patients with HIV infection have also been found to have giardiasis, the infection does not appear to be more severe among this group, regardless of the CD4+ cell count.

Immune response

Studies with humans and experimental animals 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; however, the biological role that these antibodies play in the host immune response to the infection is unclear.

IgM anti-*Giardia* antibodies are short-lived and IgG antibodies may remain at high titres for many months after the patient has been treated and cured. *Giardia*-specific IgA may also be important in both defence against and clearance of the parasite. The degree of protection of breast-fed infants against *Giardia* infection has also been shown to depend on the level of IgA-specific antibodies in breast milk. In the intestine, IgA antibodies may influence the local immune response by inhibiting parasite adherence.

Experimental animal studies provide evidence that T lymphocytes and Peyer's patch helper T lymphocytes play a role in the host immune response. In athymic mice, which are deficient in both T lymphocytes and Peyer's patch helper T lymphocytes, inoculation with *G. muris* results in a chronic infection with large number of organisms. In contrast, immunocompetent mice clear the parasite and develop resistance to reinfection.

Diagnosis

The General Stool Examination (GSE) is used primarily for this purpose. The diagnosis is based on the detection of the typical cyst in the formed stool, and the trophozoite and cyst of the parasite in diarrheal stools using normal saline and iodine preparation or using iron hematoxylin stain as in the case of *E. histolytica.*

Trophozoites must be distinguished from the non-pathogenic flagellate ***Trichomonas hominis***, which is an asymmetrical flagellate with an undulating membrane. The development of a stool enzyme-linked immunosorbent assay (ELISA) has been shown to be both a specific and sensitive rapid diagnostic tool.

Treatment

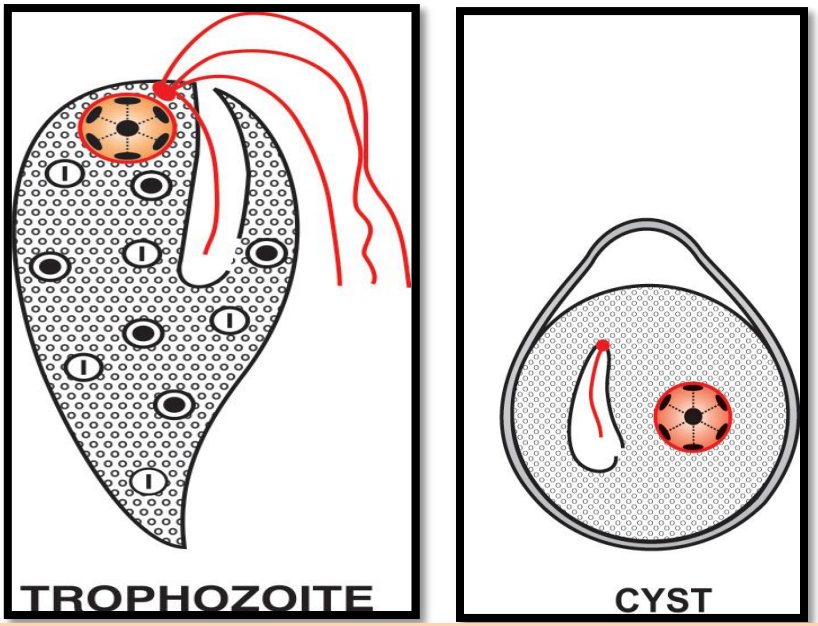
Chemotherapy with a 5-day course of *metronidazole* usually is successful, although therapy may have to be repeated or prolonged in some instances. A single dose of *tinidazole* probably is superior to *metronidazole* for treatment of giardiasis. *Paromomycin* has been used to treat pregnant women to avoid any possible mutagenic effects of other drugs.

Chilomastix mesnili

It is a common flagellate living as a harmless commensal in the caecum and the colon of man. It has a cosmopolitan distribution but is more prevalent in warm than in cool climates. It has well-defined trophozoite and cyst stages. The trophozoite is pear-shaped measuring 6-20 μm in length and 3-10 μm in breadth. It has a single nucleus lies near the center, a small distinct central karyosome and a large conspicuous cytostome (mouth) is seen on one side of the nucleus. There are three free anterior flagella, a delicate flagellum lying within the cytostome and two that encompass the lateral margins of the cytostome.

The cytoplasm is finely granular and contains numerous food vacuoles. The cyst is lemonshaped 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 trophozoites feed on enteric bacteria and multiply by binary fission. 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 the cysts stage in the stools of an infected individual. *C. mesnili* does not produce any symptoms. The diagnosis can be made by the detection of the trophozoites and cysts in the fecal smear.



Morphological forms of Chilomastix mesnili.

TRICHOMONAS

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

1. *T. tenax*

2. *T. hominis*

3. *T. vaginalis*.

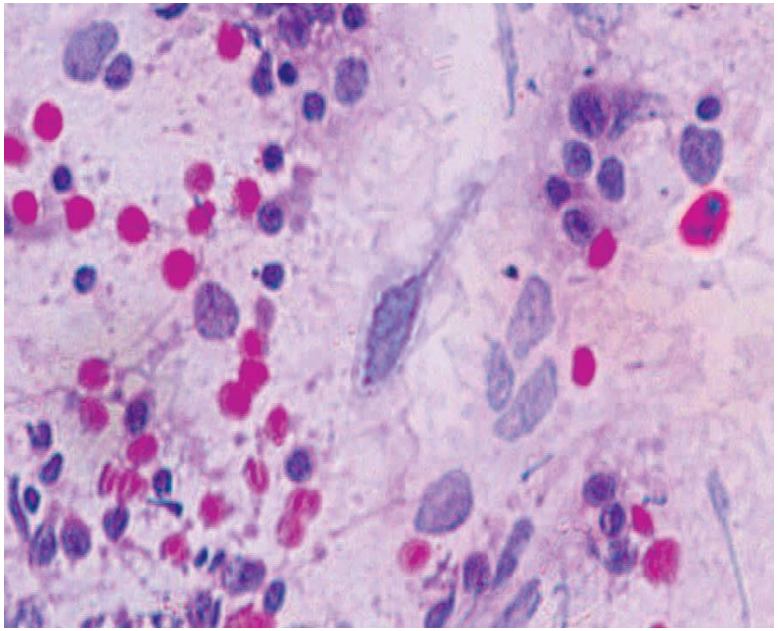
These flagellates **exist only in trophozoite stage**. Cystic stage is absent. They have four anterior flagella and one lateral flagellum which is attached to the surface of the parasite to form **undulating membrane**. The undulating membrane is supported at the base by a rod-like structure known as costa. The axostyle runs down the middle of the body and ends in the pointed tail-like extremity. A round nucleus is located in the anterior portion.

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 (Figs. 4.4 and 4.5). In a wet mount under the low-power objective the trophozoites show jerky movement. Highpower examination may reveal the beating flagella and undulating membrane characteristic of the species.



Trophozoites of Trichomonas vaginalis in vaginal smear (Papanicolaou stain, × 400).

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 and symptoms

The parasite lives on the mucosa feeding on bacteria and leucocytes. *T. vaginalis* is an **obligate parasite**. It cannot live without close association with the vaginal, urethral or prostatic tissues. Human trichomoniasis is a **widely prevalent sexually transmitted disease of worldwide importance**. Approximately 10% of vulvovaginitis is due to infection with *T. vaginalis*. Asymptomatic infections have been observed in 50% of infected female patients.

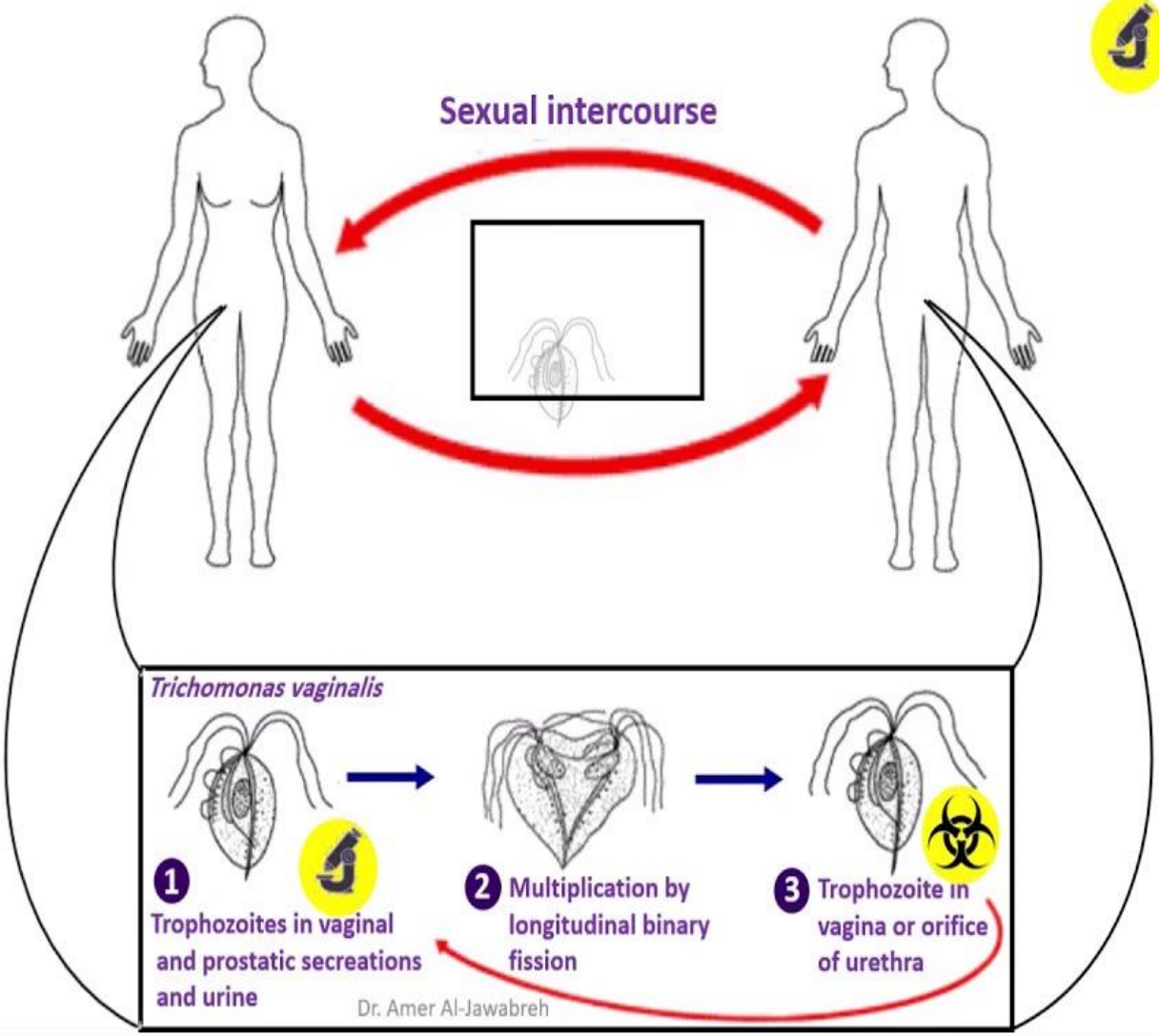
The organism is responsible for a **mild vaginitis with discharge**. Vaginal discharge contains a large number of parasites and leucocytes and is liquid, greenish or yellow. It covers the mucosa down to the urethral orifice, vestibular glands and clitoris.

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. It has been postulated that, in male patients, high concentration of zinc in prostatic fluid in urogenital tract may have lytic effect on the parasite.

The exact mechanism of pathogenesis is still not elucidated and appears to be multifactorial depending upon the inherent virulence of the parasite and host factors. 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. Presence of double-stranded RNA virus in few strains has been reported and the parasite is able to undergo phenotypic variation in its presence.

Trichomoniasis

Trichomonas vaginalis



DPDx CDC

Diagnosis

Microscopic examination of wet mounts may establish the diagnosis by detecting the actively motile trophozoites. For the diagnosis of male infections, smears of urethral and prostatic discharges stained with Giemsa stain are useful, whereas vaginal and urethral discharges in the females are the useful samples. Examination of the urine of both sexes and examination of prostate secretions of the male following prostate massage is also helpful diagnostic procedures. The parasite may also be detected by fluorescent microscopy by staining with fluorescein-labelled monoclonal antibody.

In difficult cases, cultivation of a swab sample in **Diamond's medium** can be used, but the results are not available before 3 to 7 days. Trophozoites must be distinguished from the non-pathogenic flagellate ***Trichomonas hominis***. Several types of ELISA have been developed either to measure antibodies or to detect the antigens of the parasite in clinical samples. Nucleic acid hybridization methods for the 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.

Treatment

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

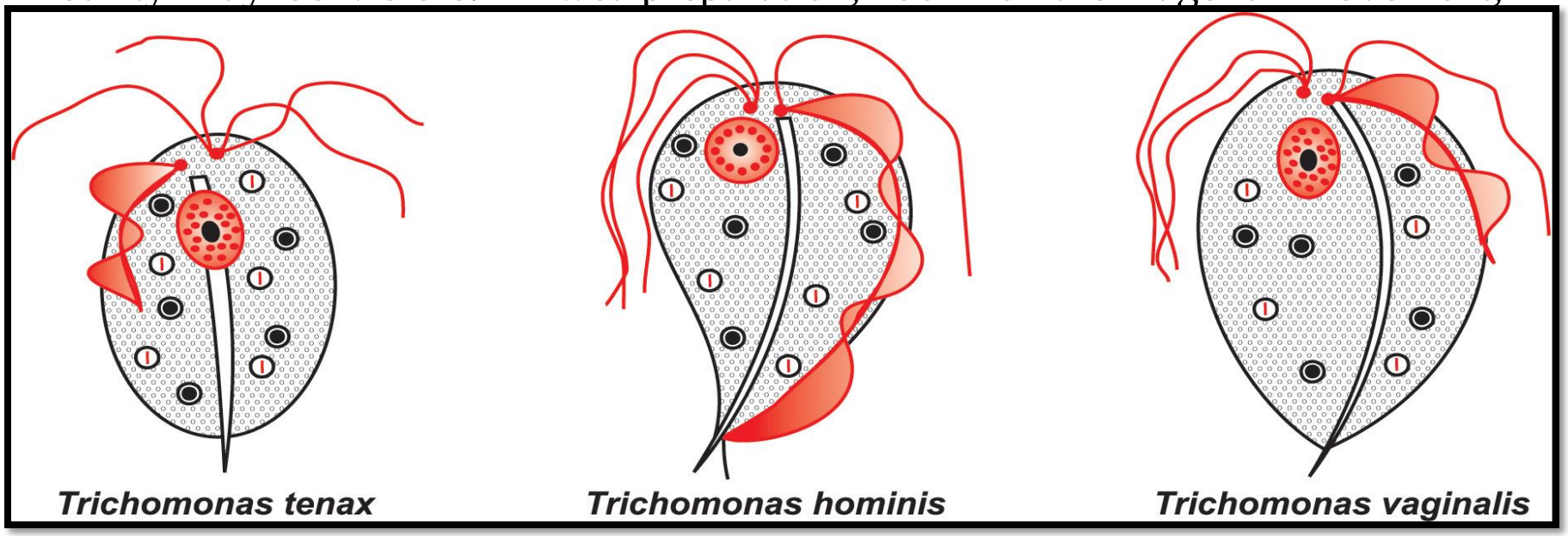
TRICHOMONAS TENAX

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. The majority of these cases have been reported from Western Europe.

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 7–10μm in width (Fig. 4.4). **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,



Trophozoites of Trichomonas spp.

Ciliates

They constitutes a group related to Sub-phylum Ciliophora. They characterized by having numerous cilia in at least one stage of their life cycle. The only ciliate that is a parasite of man is ***Balantidium coli***.

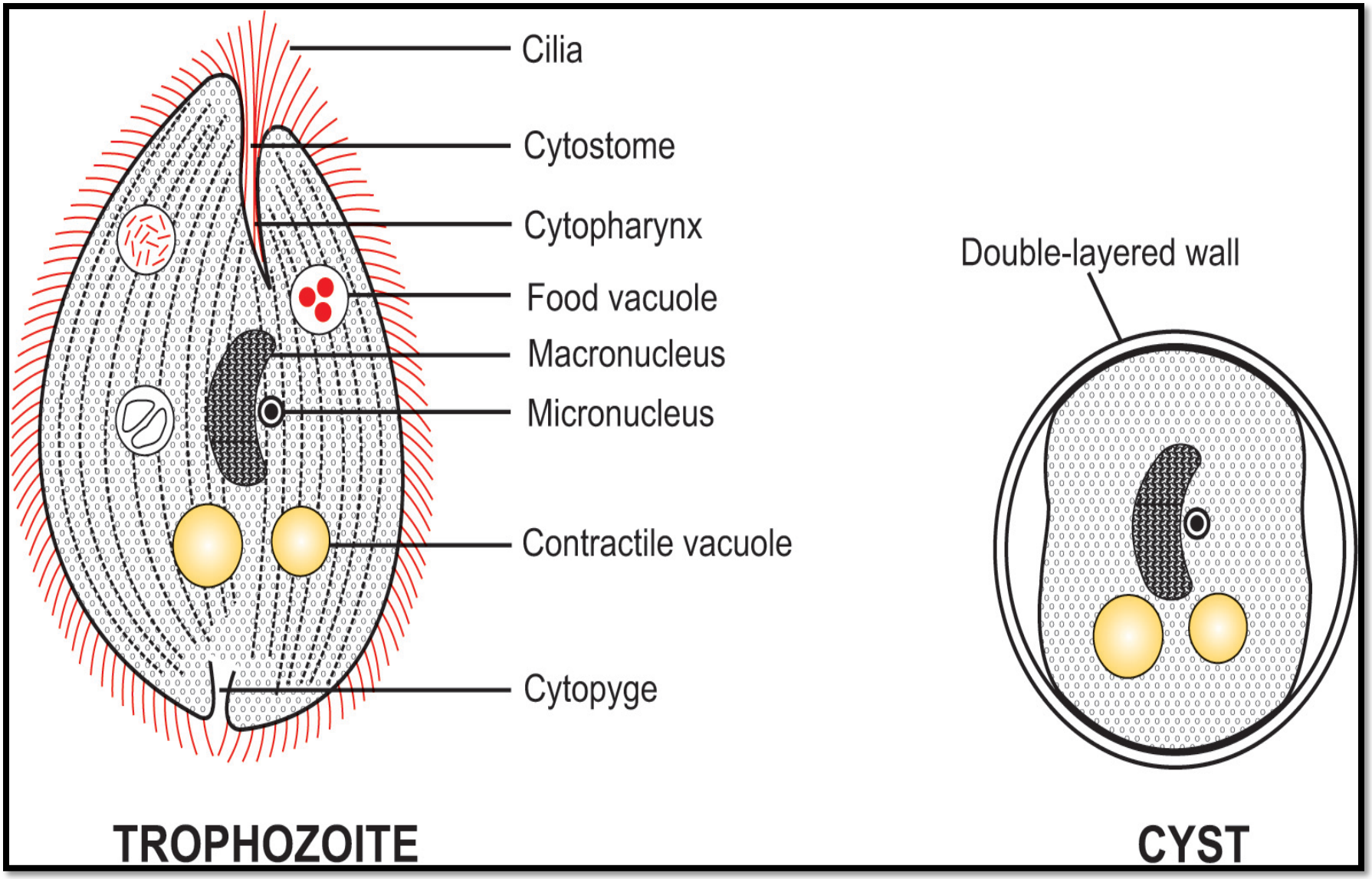
Balantidium coli

This parasite has a cosmopolitan distribution and infects the hogs, several species of monkeys as well as man. This parasite is mostly found in warm climates and tropical regions; however, its infection rate is less than 1%. It has two stages, trophozoite and cyst. The trophozoite lives in the lumen of the cecum and colon, feeding on the host cells, bacteria, food substances in the tissue or the intestine contents.

The trophozoite is the largest protozoon parasite that parasitizes man. It measures 60×45μm or more. It is ovoid, covered with a delicate pellicle showing longitudinal striations embedded in the pellicle are short cilia of relatively uniform length that are constantly in motion during life.

The anterior end is somewhat pointed and has a groove (peristome) leading to a mouth (cytostome) terminating in a short funnel-shaped gullet(cytopharynx) extending up to anterior one-third of the body.

The posterior end is broadly rounded and has an excretory opening known as cytopyge through which the residual contents of food vacuoles empty periodically. One and sometimes two larges slowly pulsating contractile vacuoles lie in the cytoplasm. The presence of contractile vacuoles, unique among parasitic protozoa, indicates a degree of osmoregulatory capability. There are also many food vacuoles in the endoplasm. Food vacuoles contain debris, bacteria, starch granules, erythrocytes and fragments of host epithelium.



Morphological forms of Balantidium coli.

The trophozoite has two nuclei, the first one lying somewhat behind the equator of the organism; it is a large kidney-shaped macronucleus and lying along the lesser curvature of the macronucleus a minute micronucleus. The parasite is pathogenic; the movement of cilia and enzyme secretions help the parasite to penetrate the mucosa. Its movement causes a rapid destruction of the tissues. It invades the mucosa and sub-mucosa of the large intestine but unlike ***E. histolytica*** because it doesn't invade the muscular coats. Multiplication is by binary fission.

Transmission of *B. coli* from one host to another is accomplished via the cyst ingested by the host. The cyst is round, measures about 40-60μ in diameter. Cilia, the large macronucleus, and contractile vacuoles are readily visible within the cyst. Encystation occurs in the small intestine and multiplication occurs in the large intestine, but may also occur outside the body of the host. From each cyst, a single trophozoite is formed. The cyst discharges with the stool of the human and hogs. The trophozoite does not encyst outside the body and disintegrates.

Pathogenicity and Symptoms

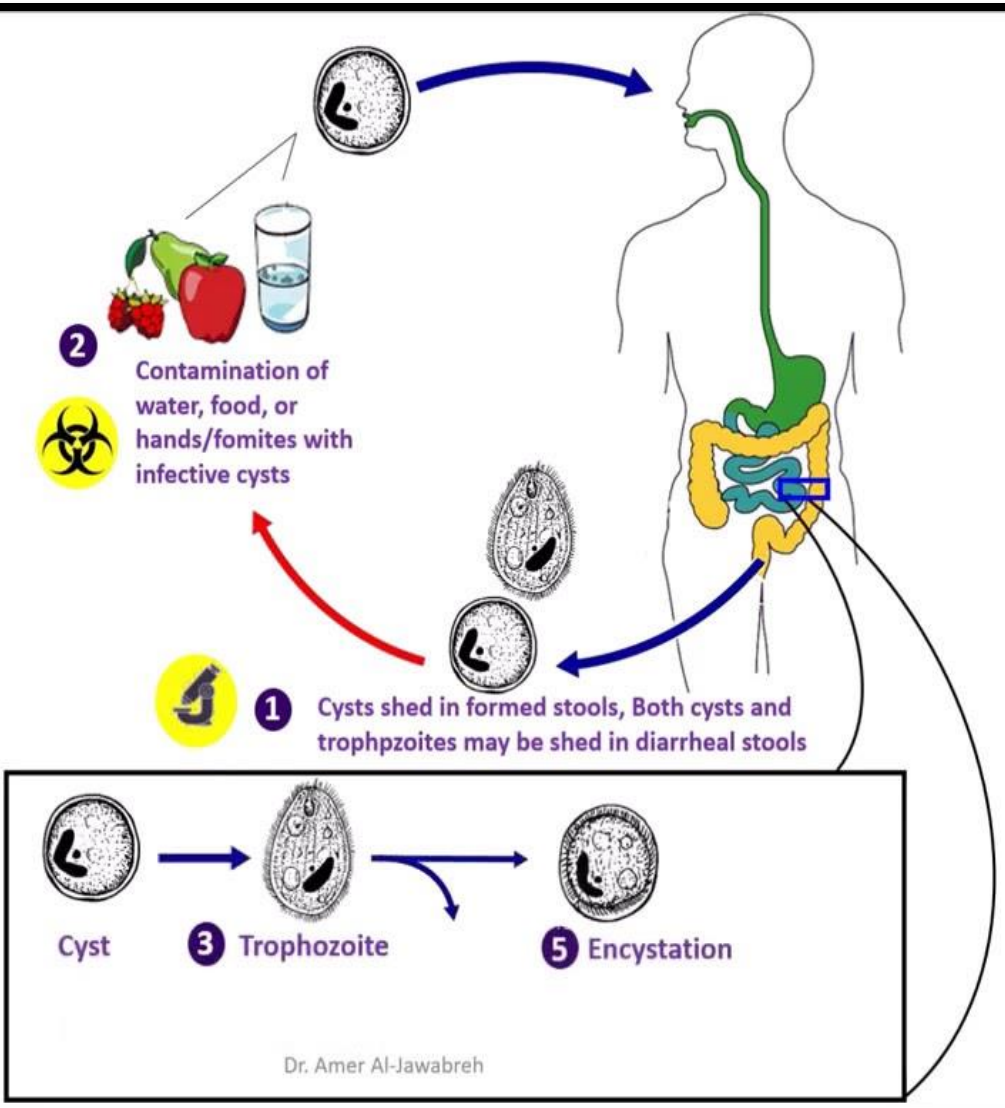
Most infections with *B. coli* are apparently harmless. The trophozoite resides in the caecal area and throughout the large intestine. It flourish in an environment rich in starch, such as the small intestine; however, in such an environment, the trophozoite does not invade the intestinal mucosa. This tendency for carbohydrates may be the reason for the trophozoite's invasive character once it becomes established in the human caecal region, a region low in carbohydrate content. It is believed that the trophozoite secretes proteolytic enzymes that act upon the mucosal epithelium, facilitating the invasion. The ulcers are round, ovoid, or irregular in shape with undermined edges. The floor of the ulcer is covered with pus and necrotic material. The abscesses are usually small and when incised are found to be filled with a mucoid material containing numerous Balantidia. The intervening mucosa may or may not be inflamed.

On microscopic examination, parasites are frequently seen in clusters in the submucosa or at the bases of the crypts. They can easily be recognized because of the presence of the macronucleus which stains deeply with hematoxylin and eosin. The symptoms vary from acute to asymptomatic carrier state. Parasitic invasion of the mucosal epithelium is followed by hemorrhage and ulceration, hence the name balantidial dysentery often given to this condition. While symptoms such as colitis and diarrhea may resemble amoebiasis in many respects, extra intestinal disease is rare.

The symptoms represented by fulminating dysentery or acute diarrhea; dysenteric feces consist of mucus, blood, pus, the diarrhea 6-15 times daily. The chronic infection causes general weakness, chronic diarrhea, nausea, abdominal pain and anemia. Chronic recurrent diarrhea alternating with constipation is the most common clinical manifestation, but there may be bloody mucoid stools, anorexia, nausea, epigastric pain, vomiting and intestinal colic.

Balantidiasis

Balantidium coli



=Infective stage

=Diagnostic stage

DPDx

CDC

Diagnosis

The usual diagnostic procedure consists of stool examination for the presence of trophozoites and cysts. Cysts can be identified by their large size, heavy cyst wall, large macronucleus, and the presence of cilia within the cyst whereas the trophozoites are readily identified by their large size and the fact that *B. coli* is the only ciliophoran parasite in humans. Diagnosis can also be made by the examination of biopsy specimens taken with the help of a sigmoidoscope or by examination of scrapings of an ulcer.

Treatment

Tetracycline 500 mg four times a day for 10 days or metronidazole 750 mg three times a day may be used for the treatment of *B. coli* infection.