



Introduction

GENERAL CHARACTERS:

- 1- Movement of the flagellates is accomplished by the presence of flagella in their trophozoite form.
- 2- All flagellate life cycles consist of the trophozoite form. Cysts, are not known to exist in several of the flagellate life cycle.
- 3- In those flagellate life cycles with no known cyst stage, the trophozoite is considered to be more resistant to destructive forces, surviving passage into the stomach following ingestion. In addition, these trophozoite also appear to survive outside the environment.
- 4- In flagellate life cycles that consist of both trophozoite and cyst, the processes of encystation and excystation occur.
- 5- Unlike the amoebae, flagellates reside mainly in the small intestine, cecum, colon, and the case of *Giardia lamblia*, the duodenum.
- 6- The flagellate cysts, like those of the amoebae, are equipped with thick, protective cell wall.
- 7- Flagellates possess one advantage over their amoeboid relatives in that they can swim. Therefore, enabling them to invade and adapt to a wider range of environments unsuitable for other amoebae. They are able to change from a flagellated free-swimming environment to a non-flagellated tissue dwelling stage and *vice versa*
- 8- Flagellates are known to inhabit the reproductive tract, alimentary canal, tissue sites and also the blood stream, lymph vessels and cerebrospinal canal.

CLASSIFICATION

Flagellates are divided in two groups:

- 1- Kinetoplastida: They possess a kinetoplast from which arises a single flagellum. This group includes *Trypanosomes* and *Leishmania* which transmitted to the human by insect and cause systemic or local infections.
- 2- Flagellates without kinetoplast: they have multiple flagella such as *Giardia lamblia*, *Trichomonas* and other intestinal flagellates .

The flagellates may be classified according to their habitat into:

- 1- Intestinal, oral and genital flagellates. This includes the following parasite with their habitat:

- *Giardia lamblia* (duodenum, jejunum)
- *Trichomonas vaginalis* (vagina, urethra)
- *Trichomonas tenax* (mouth)
- *Trichomonas hominis* (caecum)
- *Chilomastix mesnili* (caecum)
- *Enteromonas hominis* (colon)
- *Retortamonas intestinalis* (colon)
- *Dientamoeba fragilis* (colon)

Except *Giardia lamblia* and *Trichomonas vaginalis* all are non-pathogenic.

2- Blood and tissue flagellates (Haemoflagellates): These flagellates infect the vascular system and various tissues of the body. Two important genera pathogenic to man are:

- *Trypanosomes*
- *Leishmania*

INTESTINAL FLAGELLATES

***Giardia lamblia*:**

Giardia lamblia is a flagellate of world-wide distribution. It is more common in warm climates than temperate climates. It is the most common flagellate of the intestinal tract, causing *Giardiasis*. Humans are the only important reservoir of the infection. The infection is most common in parts of the world where sanitation is at its lowest. *Giardiasis* is an infection of the upper small bowel, which may cause diarrhea

Morphology

Cyst : are non-motile and egg-shaped. They measure 8–14 μm by 7–10 μm . The cysts are encased by a smooth and colorless, thick and refractile wall. Immediately after encystation, newly formed cysts contain two genetically identical nuclei. Each organelle duplicates so that in permanently stained mature cysts, four prominent nuclei and four median bodies are observed. Compared to trophozoites, cysts also have twice the number of intracytoplasmic flagellar structures. The cysts are the infective form of the parasite and each cyst gives rise to two binucleated trophozoites which are motile and non-infectious because they cannot survive long outside the host body.

Trophozoites: are pear shaped with a broad anterior end and a narrow posterior end. It is 9–21 μm long and 5–15 μm wide. The parasite is bilaterally symmetrical and dorsoventrally flattened. A large sucking disk, which allows the parasite to attach to the surface of the intestinal mucosa of the host, takes up most of the ventral surface of the parasite. Behind the sucking disks, two rods known as median bodies are seen. Four pairs of flagella are located anterior, lateral, ventral, and posterior on the body of the organism. The pair of anterior flagella, known as axoneme, is straight, closely approximated and parallel to each other, dividing the body of the organism into two halves longitudinally. Motility brought by the four pairs of flagella is essential for virulence of the parasite. The two spherical or ovoid nuclei, containing a large, central karyosome, can be found on each side of the axonemes. The parasite does not have peripheral chromatin.

Life cycle

life cycle of *Giardia* alternates between the cyst and the trophozoite forms, and both forms are found in feces. Cysts are more often found in non-diarrheal feces, and they are the infectious stage of the parasite. The cysts are hardy and resistant to standard concentrations of chlorine used in water treatment and they can persist for several months in cold, moist environment.

Infection begins when a new host ingests cysts in contaminated food, water, fomites or fecal-orally. Mature cysts are able to survive the acidic environment of the stomach and migrate to the small intestine of the host. Exposure to stomach acid triggers a process called excystation, during which trophozoites are released from cysts. Each quadrinuclear cyst gives rise to two binuclear trophozoites. The trophozoites multiply asexually by binary fission in the small intestine, either as free floating bodies or attached to the intestinal epithelium. Trophozoites are the disease causing stage of the parasite and they colonize the small intestine by attaching to the intestinal mucosa using the ventral sucking disks. Trophozoites are largely noninvasive and do not invade other organs; however, at times they might penetrate down into the secretory tubules of the mucosa and be found in gallbladder and the biliary drainage. As trophozoites migrate toward the large intestine, they retreat into the cyst form in a process called encystation. Bile salts and intestinal mucous were found to enhance trophozoite multiplication and encystations. Trophozoites, if excreted in feces, cannot survive long in the environment and are therefore noninfectious. The cysts in excrements will quickly become infectious and will begin a new cycle of infection if ingested by a new host.

pathogenesis

Colonization of the gut results in inflammation and villous atrophy, reducing the gut's absorptive capability. In humans, infection is symptomatic only about 50% of the time, and protocol for treating asymptomatic individuals is controversial. Symptoms of infection include diarrhea, malaise, excessive gas (often flatulence or a foul or sulphuric-tasting belch, which has been known to be so nauseating in taste that it can cause the infected person to vomit), steatorrhea (pale, foul smelling, greasy stools), epigastric pain, bloating, nausea, diminished interest in food, possible (but rare) vomiting which is often violent, and weight loss. Pus, mucus and blood are not commonly present in the stool. In healthy individuals, the condition is usually self-limiting, although the infection can be prolonged in patients who are immunocompromised, or who have decreased gastric acid secretion.

People with recurring *Giardia* infections, particularly those with a lack of IgA, may develop chronic disease. Some studies have shown that giardiasis should be considered as a cause of vitamin B₁₂ deficiency, this a result of the problems caused within the intestinal absorption system.

Diagnosis

The patient's history may indicate recent exposure to *Giardia*, but the infection is diagnosed, as in most parasitic infections, by identifying the organism. In the case of giardiasis, cysts are found in formed stool. Diarrheal specimens may also contain trophozoites. If still motile, the trophozoites exhibit a typical “falling leaf” movement.

Because cysts are often shed intermittently, three stool specimens should be obtained at approximately 48-hour intervals. The chance of finding cysts in a light infection increases if the stool specimen is subjected to a concentration method, such as the zinc sulfate centrifugal flotation technique.

Other tests that can be used for diagnosing giardiasis are collection and examination of fluid from the duodenum or biopsy of the small intestine, but these require tests that involve expense and discomfort. The string test is a more comfortable method for obtaining a sample of duodenal fluid. For the string test, a gelatin capsule that contains a loosely-woven string is swallowed. One end of the string protrudes from the capsule and is taped to the patient's outer cheek. Over several hours, the gelatin capsule dissolves in the stomach, and the string uncoils, with the last 12 inches or so passing into the duodenum. In the duodenum the string absorbs a small amount of duodenal fluid. The string then is untaped from the cheek and is removed. The collected duodenal fluid is expressed from the string and is examined under the microscope.

The best test for diagnosing giardiasis is antigen testing of the stool. For antigen testing, a small sample of stool is tested for the presence of *Giardia* protein. The antigen test will identify more than 90% of people infected with *Giardia lamblia*.

Serological methods of diagnosis are proving to be useful as mean of diagnosis. An ELISA to detect IgM in serum provides evidence of a current infection.

Treatment

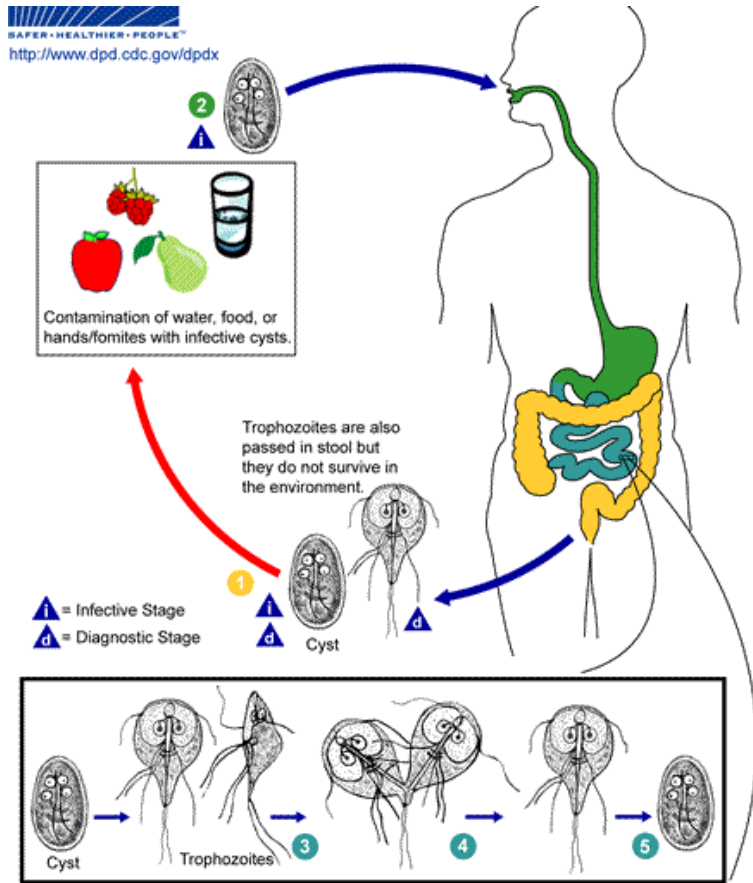
Human infection is conventionally treated with metronidazole , tinidazole or nitazoxanide

Epidemiology

Giardia infection occurs worldwide, with an incidence usually ranging from 1.5 to 20 percent. Higher incidences are likely where sanitary standards are low. Although people of all ages may harbor these organisms, infants and children are more often infected than are adults. Carriers are probably more important in the spread of these organisms than symptomatic patients because cysts are less likely to be present in diarrheic stool. Like other diseases spread by the fecal-oral route, giardiasis can be a problem in institutions, nurseries, and day-care centers.. The outbreaks have been caused by drinking contaminated water from community water supplies or directly from rivers and streams.

Prevention

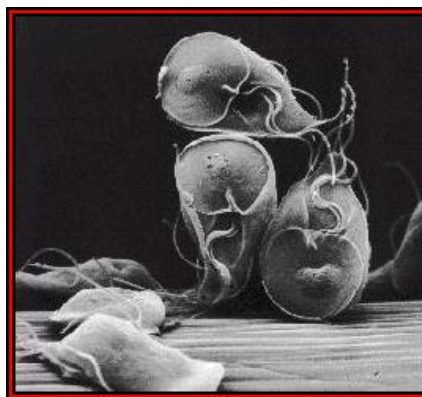
Attention to personal hygiene is the key to preventing the spread of giardiasis. Controlling the spread of *Giardia* in drinking water should be possible where community water treatment methods (e.g., disinfection and filtration) are available. Chlorine kills *Giardia* cysts under appropriate conditions. Boiling promptly inactivates *Giardia* cysts and is the best solution.



Life cycle of *Giardia lamblia*



Cyst of *Giardia lamblia*



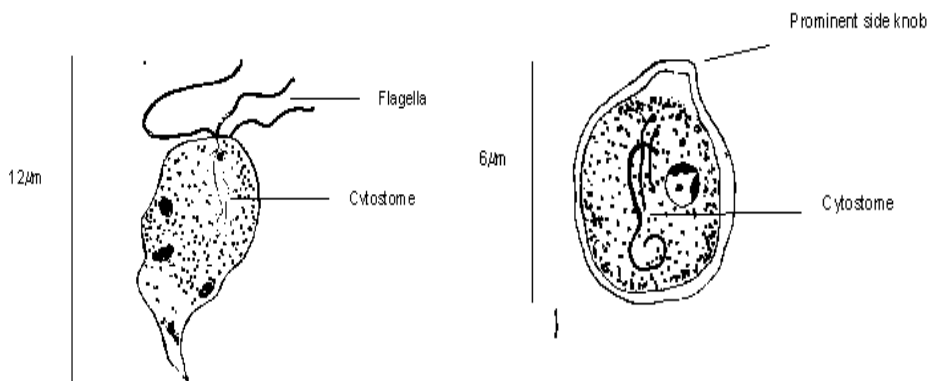
Troph. Of *Giardia Lamblia*

Chilomastix mesnili

Chilomastix mesnili is of cosmopolitan distribution although found more frequently in warm climates. It is thought to be non-pathogenic although the trophozoite has been associated with diarrhoeic stool.

Morphology

The cyst is 6-9µm. It has a large single nucleus with a large karyosome. It has a prominent side knob which gives it a characteristic lemon shape. The cytosome is evident with a curved shepherd's crook fibril.



The trophozoites of *C. mesnili* are pear shaped and measure 10-20µm in length. It has one large nucleus with a small karyosome and 3 flagella which extend from the nucleus at the anterior end of the parasite. A distinct oral groove or cytosome can be seen near the nucleus. It moves in a directional manner.

Laboratory diagnosis

The characteristic lemon shaped cysts can be seen in a formol-ether concentrate. Motile organisms can be seen in a wet preparation of a fresh stool however the characteristic morphology is evident in a permanently stained preparation.

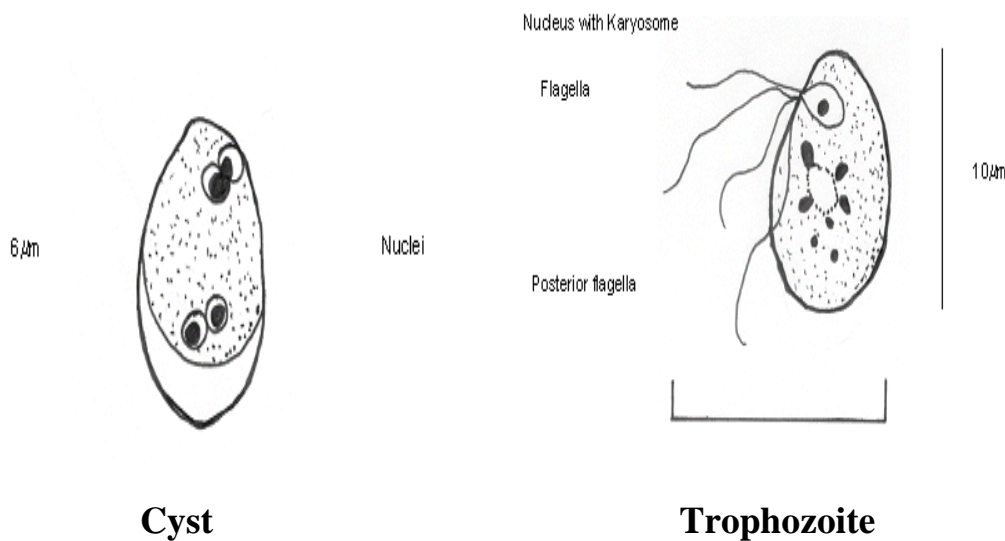
Enteromonas hominis

Enteromonas hominis is a small flagellate and is rarely encountered. It is found in both warm and temperate climates and is considered to be non-pathogenic.

Morphology

The cyst is oval and is 6-8µm in length. It has up to four nuclei with a bipolar tendency.

The trophozoite is oval and 4-10µm in length. It has a jerky rotation. It has 4 flagella, 3 anterior flagella and one adheres to the body ending in a tail. It has one nucleus with a large karyosome which is evident in a stained preparation.



Laboratory Diagnosis

The cysts are seen in a formol-ether concentrate. The cysts have no distinguishing characteristics and thus can be confused with *E. nana* or even yeasts. The characteristic trophozoites can be seen in a permanently stained faecal smear.

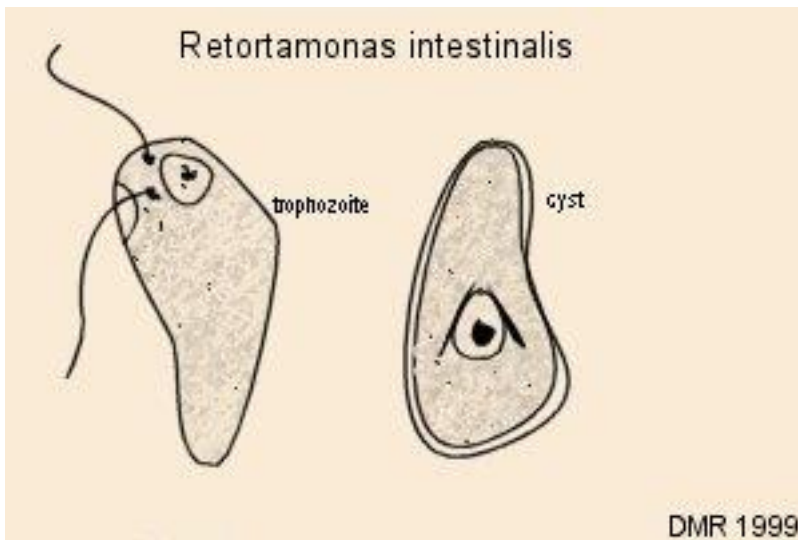
Retortamonas intestinalis

Retortamonas intestinalis like *Enteromonas hominis* is a small flagellate found in both warm and temperate climates and is considered to be non-pathogenic.

Morphology

The cyst is small and pear shaped. It is 4-7µm with one large nucleus frequently near the centre. The fibril arrangement from the nucleus is suggestive of a birds beak. This is characteristic.

The trophozoite is small, measuring between 4 and 9µm. Its movement is jerky and rotational and has 2 anterior flagella and a prominent cytosome which can be seen in an unstained preparation. It has a relatively large nucleus at the anterior end with a small compact karyosome.



Trophozoite

Cyst

Laboratory Diagnosis

The small pear shaped cysts are uncharacteristic in an unstained formol-ether preparation. However, the addition of iodine reveals the characteristic bird beak fibrillar arrangement in the pear shaped cyst.

In a fresh stool preparation, the 2 anterior flagella and cytosome can be seen in the trophozoite. In a permanently stained preparation, the large nucleus with small central karyosome is diagnostic

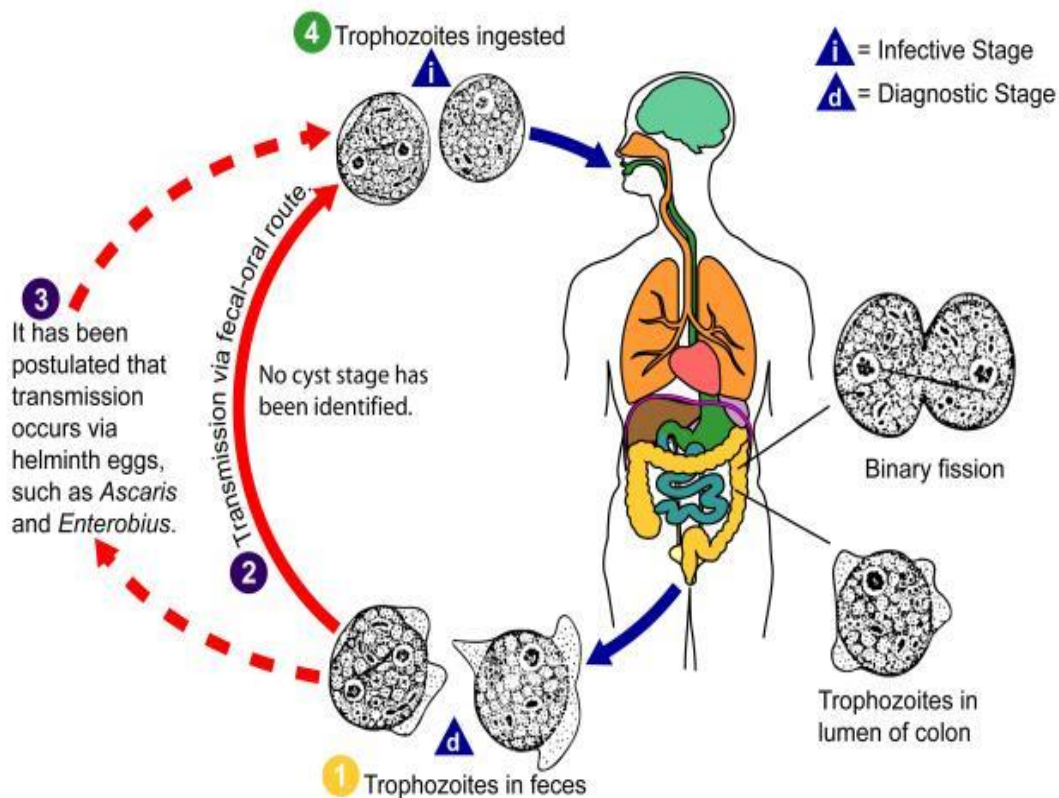
Dientamoeba fragilis

Morphology of Trophozoites and Life cycle

D. fragilis are relatively small, varying from 3-22mm in diameter and there can be considerable variation in size among organisms in the same fecal sample. The organisms have only a trophozoite stage and in a permanently stained preparation, one, two or rarely three nuclei can be seen, two being the most common. The nuclear chromatin is usually fragmented into three to five granules but these have not been visualized by Giemsa Stain, and there is normally no peripheral chromatin on the nuclear membrane. The cytoplasm is usually vacuolated and may contain ingested debris as well as some large uniform granules. The cytoplasm can also appear uniform and clean with a few inclusions. *D. fragilis* live in the lumen of the cecum and upper colon.

Dientamoeba fragilis Infection

(*Dientamoeba fragilis*)



Pathogenesis

Historically *Dientamoeba* has been considered as a non-pathogenic commensal. However, clinical symptoms often correlate with the presence of large numbers of trophozoites and treatment of the infection resolves the symptoms. The incidence of symptoms is estimated at 15-30% of infected individuals. Clinical symptoms associated with *Dientamoeba* include intermittent diarrhea, abdominal pain, flatulence, nausea and fatigue. Little is known about the pathogenesis and *Dientamoeba* probably acts as a low-grade irritant of intestinal mucosal surfaces that may lead to some inflammation. Iodoquinol is generally the drug of choice for the treatment of *Dientamoeba*. Tetracycline, paromomycin, and metronidazole are also effective.

Diagnosis

Diagnosis is dependent on examination of the fresh direct wet preparation or permanently stained smears prepared from unformed or formed stools with mucus. It is particularly important that permanently stained smears of stool preparations should be examined, because survival times of the organism in terms of morphology, is very limited and specimens must be examined immediately or preserved in a suitable fixative as soon as possible after defecation. The recommended stains are Fields' and Giemsa stain.

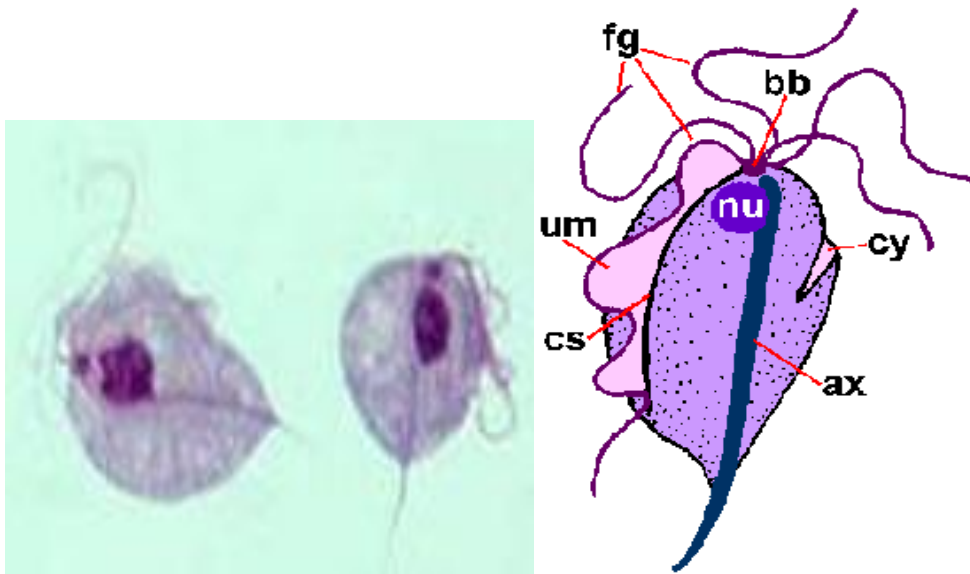
Trichomonadidea:

- 1- *Trichomonas vaginalis*
- 2- *Trichomonas tenax*
- 3- *Trichomonas hominis*

Trichomonas vaginalis

Morphology

T. vaginalis trophozoite : is oval as well as flagellated. It is slightly larger than a white blood cell, measuring 9 X 7 μm . Five flagella arise near the cytostome; four of these immediately extend outside the cell together, while the fifth flagellum wraps backwards along the surface of the organism. In addition, a conspicuous barb-like axostyle projects opposite the four-flagella bundle; the axostyle may be used for attachment to surfaces and may also cause the tissue damage noted in trichomoniasis infections. The cytoplasm shows prominent granules which are most numerous alongside the axostyle and costa. The trophozoite has short undulating membrane reaching up to the middle of the body. While *T. vaginalis* does not have a cyst form, organisms can survive for up to 24 hours in urine, semen, or even water samples. It has an ability to persist on fomites with a moist surface for 1 to 2 hour.



Trophozoite of *Trichomonas vaginalis*

Pathogenesis and symptoms

Trichomoniasis is a common sexually transmitted disease with a worldwide distribution and an estimated 167 million people becoming infected per year worldwide. Trichomoniasis is believed to be the most common non-viral sexually transmitted disease. However it is now recognized a factor in promoting HIV infection causing low-weight and premature births, and predisposing women to substantial discomfort and stress.

The pathology caused by *Trichomonas* may enhance the efficiency of HIV transmission . *T. vaginalis* infection typically elicits a local cellular immune response with inflammation of the vaginal epithelium and cervix in women and the urethra of men. This inflammatory response includes the infiltration of potential HIV target cells such as CD4+ bearing lymphocytes and macrophages. In addition, *T. vaginalis* can cause punctate hemorrhages on the vaginal walls and cervix. This leukocyte infiltration and the genital lesions may increase the number of target cells for the virus and allowing direct viral access to the bloodstream through open lesions. In addition, the hemorrhages and inflammation can increase the level of virus in body fluids and the numbers of HIV-infected lymphocytes and macrophages present in the genital area in persons already infected with HIV.

T. vaginalis, despite its name, infects both men and women. In females the organism primarily inhabits the vagina, and in males it is usually found in the urethra, prostate or epididymis. The life cycle consists only of a trophozoite stage which is transmitted by direct contact during sexual intercourse. Non-venereal transmission is rare, but possible since the trophozoites can survive 1-2 days in urine and 2-3 hours on a wet sponge. In addition, neonatals have been infected during the birth process. The trophozoites live closely associated or attached to the epithelium of the urogenital tract, where they replicate by binary fission.

T. vaginalis causes different clinical manifestations in men and women and women are more likely to exhibit symptoms which tend to persist longer. The incubation period typically ranges from 4-28 days. In females the infection can present as a mild vaginitis, an acute or chronic vulvovaginitis, or urethritis. The onset or exacerbation of symptoms commonly occurs during or immediately after menstration. The most common complaint associated with *T. vaginalis* infection is a persistent mild vaginitis associated with a copious, foul-smelling discharge that is often accompanied by burning or itching. This discharge is most often gray, but can be yellow or green and is occasionally frothy or blood tinged. The discharge diminishes as the infection becomes more chronic. Urethral involvement occurs in a large number of cases and is characterized by dysuria (painful urination) and frequent urination.

The vaginal epithelium is the primary site of infection. Thus the vaginal walls are usually erythematous and may show petechial (a small non-raised spot) hemorrhages. Punctate hemorrhages of the cervix, called strawberry cervix, are observed in approximately 2% of the cases. This strawberry cervix is a distinctive pathological observation associated with trichomoniasis not seen with other sexually transmitted diseases.

Males are likely to be asymptomatic (50-90%) and the infection tends to be self-limiting. The urethra and prostate are the most common sites of infection. Common symptoms include: urethral discharge (ranging from scant to purulent), dysuria, and urethral pruritus (itching).

DIAGNOSIS, TREATMENT AND CONTROL

- Diagnosis is confirmed by the demonstration of trophozoites in vaginal, urethral, prostatic secretions, or urine sediment (following prostate massage).
- **Microscopic examination** of wet mounts of fresh vaginal discharge, preferably collected with a speculum on a cotton-tipped applicator, is the most practical method of diagnosis. Specimens should be diluted in saline and examined immediately. *T. vaginalis* is recognized by its characteristic morphological features and its rapid jerky motility.
- Specimens can also be fixed and stained with Giemsa or fluorescent dyes. However, the organism may be difficult to recognize on stained slides.
- **Culture** is considered the gold standard for diagnosis. Culture system called Pouch™TV culture system. This is a commercially available self-contained system for the detection of *T. vaginalis* in clinical specimens.
- **Antibody and DNA-based** tests with high sensitivity and specificity are being developed.

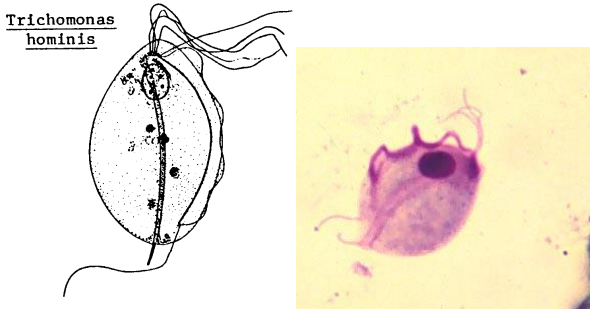
Metronidazole (Flagyl®) and other nitroimidazoles, such as tinidazole, are highly effective against trichomoniasis.

The epidemiology of trichomoniasis exhibits features similar to other sexually transmitted diseases (STD) and incidence correlates with the number of sexual partners. In addition, co-infection with other STDs is common. It is estimated that up to 25% of sexually active women will become infected at some point during their lives and the disease will be transmitted to 30-70% of their male partners. Measures used in the control of other STD, such as limiting number of sexual partners and use of condoms, are also effective in preventing trichomoniasis.

Trichomonas hominis

Morphology.

T. hominis has no cystic stage. The trophozoite measures from 5-15µm in length by 7-10µm in width. The shape is pyriform and has an axostyle which runs from the nucleus down the centre of the body and extends from the end of the body and undulating membrane which extends the entire length of the body and projects from the body like a free flagellum. It has four free flagella and a single nucleus at the anterior end.

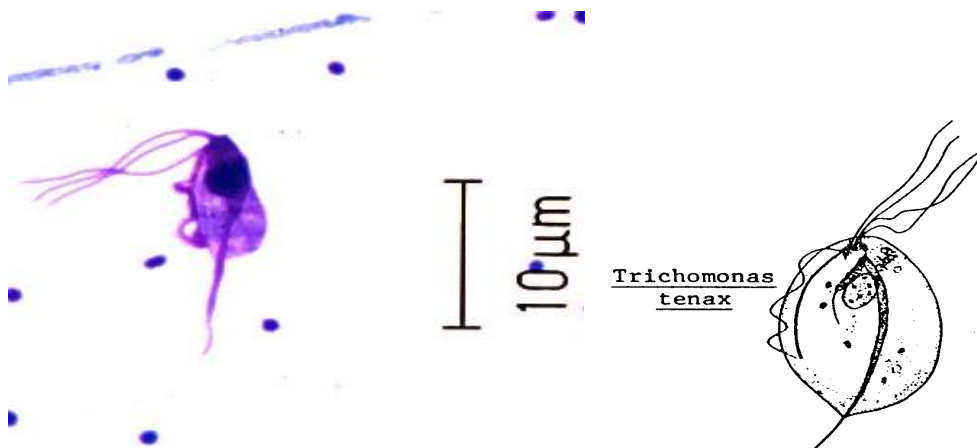


Laboratory Diagnosis

In a fresh stool, the flagellates move very rapidly in a jerky, non-directional manner. The axostyle and undulating membrane are diagnostic.

Trichomonas tenax

A species that lives as a commensal in the mouth of humans and other primates, especially in the tartar around the teeth or in the defects of carious teeth; there is no evidence of direct pathogenesis, but it is frequently associated with pyogenic organisms in pus pockets or at the base of teeth. The trophozoite is oval to pear shaped and measuring 5-14Mm long, with average length of 6 to 9Mm. The single ovoid vesicular nucleus is filled with several chromatin granules and is usually located in the center anterior portion of the organism. The *T. tenax* trophozoite is equipped with five flagella, all of which originate at the anterior end. Four of the flagella extend anteriorly and one extends posteriorly. An undulating membrane that extends two thirds of the body length and its accompanying costa typically lie next to the posterior flagellum. A thick axostyle runs along the entire body length, curving around the nucleus, and extends posteriorly beyond the body of the organism. There is no cyst stage of *T. tenax*.



Labratory Daignosis

The specimen of choice for diagnosing *T. tenax* trophozoite is mouth scrapings. Microscopic examination of tonsillar crypts and pyorrheal pockets of patients suffering from *T. tenax* infections often yields the typical trophozoites. Tartar between the teeth and the gingival margin of the gums are the primary areas of the mouth that may also potentially harbor this organism.