PATHOGENESIS AND CLINICAL SYMPTOMS

There are many similarities in terms of pathogenesis and clinical symptoms between flagellates and amebas. Although this section is written specifically about flagellates, the information covered pertains to both groups of parasites.

Flagellates are often recovered from patients suffering from diarrhea without an apparent cause. In addition, there are a number of asymptomatic flagellate infections. It is important to identify the nonpathogenic flagellates because this finding suggests the ingestion of contaminated food or drink. Pathogenic flagellates have transmission routes similar to those of the nonpathogenic variety. Careful examination of all samples, especially those containing nonpathogenic flagellates, is essential to proper identification of all possible parasites present.

It is important to note that there is only one intestinal flagellate, *G. intestinalis*, that is considered pathogenic. Infections with *G. intestinalis* may produce characteristic symptoms. Each of the atrial flagellates may cause symptoms in areas such as the mouth and genital tract.

Quick Quiz! 4-3

The presence of nonpathogenic flagellates is important because it suggests that: (Objective 4-5A)

A. The patient will develop clinical signs and symptoms.
B. Only cyst forms will be recovered in corresponding patient samples.
C. The parasites will invade multiple organ systems in the body.
D. Contaminated food or drink was consumed by the patient.

FLAGELLATE CLASSIFICATION

The flagellates belong to the subphylum Mastigophora, class Zoomastigophora. Like the amebas, the flagellates may be separated into two categories, intestinal and extraintestinal. Figure 4-1 identifies the species that fall under each category.

Giardia intestinalis
(gee'are-dee'uh/in-tes-ti-nal-is)

Common associated disease or condition names: Giardiasis, traveler's diarrhea.

Initially known as *Cercomonas intestinalis*, this important flagellate was first discovered in 1859 by French scientist Dr. F. Lambl. In honor of the significant contributions of both Dr. Lambl and Czechoslovakian scientist Dr. Giard to the field of parasitology, Stiles coined the term *Giardia lamblia* (pronounced lamb-bleé uh) in 1915 (see the Notes of Interest and New Trends section for additional historical information). Since the term *Giardia intestinalis* is gaining

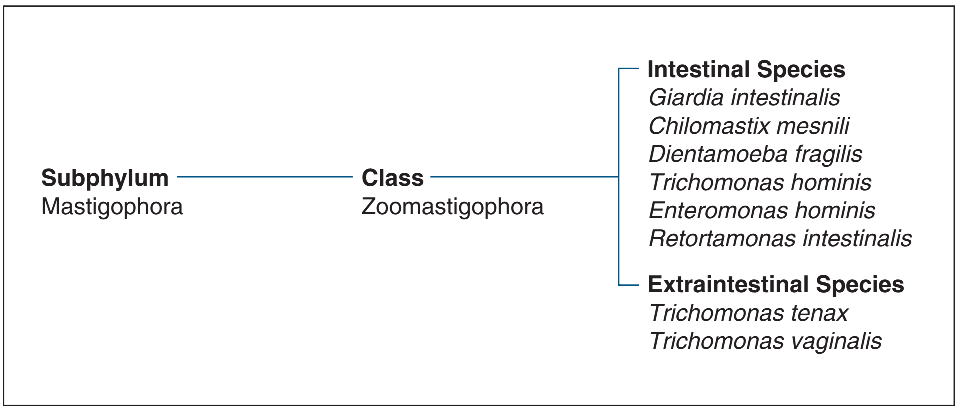
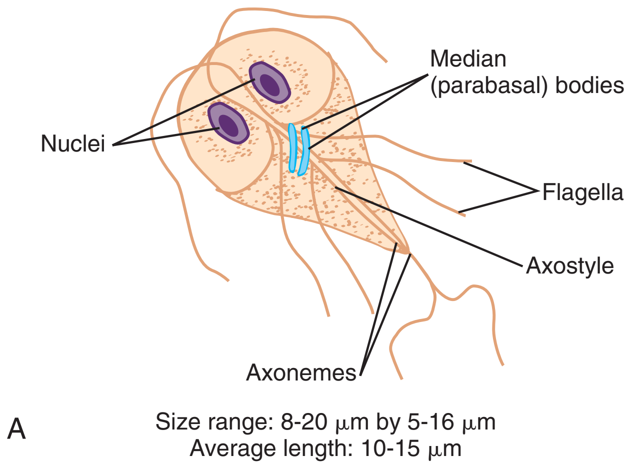


FIGURE 4-1 Parasite classification, the flagellates.



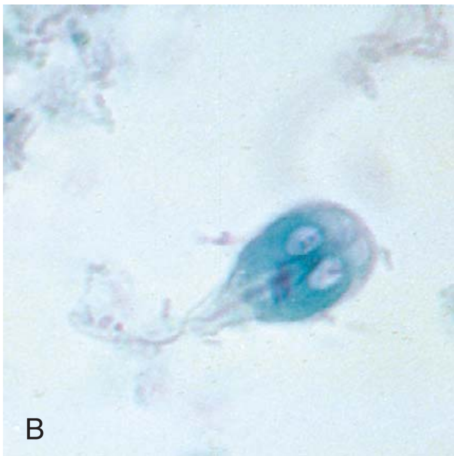


FIGURE 4-2 **A,** *Giardia intestinalis* trophozoite. **B,** *Giardia intestinalis* trophozoite. *(**B** from Forbes BA, Sahm DF, Weissfeld AS: Bailey & Scott's diagnostic microbiology, ed 12, St Louis, 2007, Mosby.)*

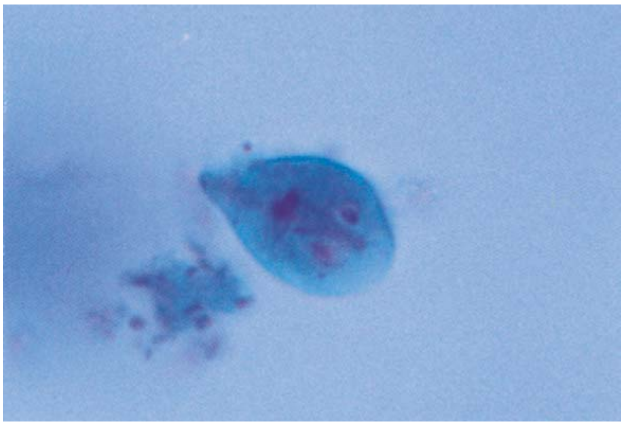


FIGURE 4-3 *Giardia intestinalis* trophozoite. Note red-staining nuclei (trichrome stain, ×1000).

TABLE 4-1 ***Giardia intestinalis* Trophozoite: Typical Characteristics at a Glance**

Parameter	Description
Size range	8-20 µm long 5-16 µm wide
Shape	Pear-shaped, teardrop
Motility	Falling leaf
Appearance	Bilaterally symmetrical
Nuclei	Two ovoid-shaped, each with a large karyosome No peripheral chromatin
Flagella	Four pairs, origination of each: One pair, anterior end One pair, posterior end Two pair, central, extending laterally
Other structures	Two median bodies Two axonemes Sucking disk

popularity (some also consider *Giardia duodenale* as a synonym), its formal name is currently under review by the International Commission on Zoological Nomenclature. For the purposes of this text, this parasite will be referred to as *Giardia intestinalis*.

Morphology

Trophozoites. The typical *G. intestinalis* trophozoite ranges from 8 to 20 µm in length by 5 to 16 µm in width (Figs. 4-2 and 4-3; Table 4-1). The average *G. intestinalis* trophozoite, however, measures 10 to 15 µm long. The *G. intestinalis* trophozoite is described as pear or teardrop shaped. The broad anterior end of the organism tapers off at the posterior end. The *G. intestinalis* trophozoite characteristically exhibits motility that resembles a falling leaf. The trophozoite is bilaterally symmetrical, containing two ovoid to spherical nuclei, each with a large karyosome,

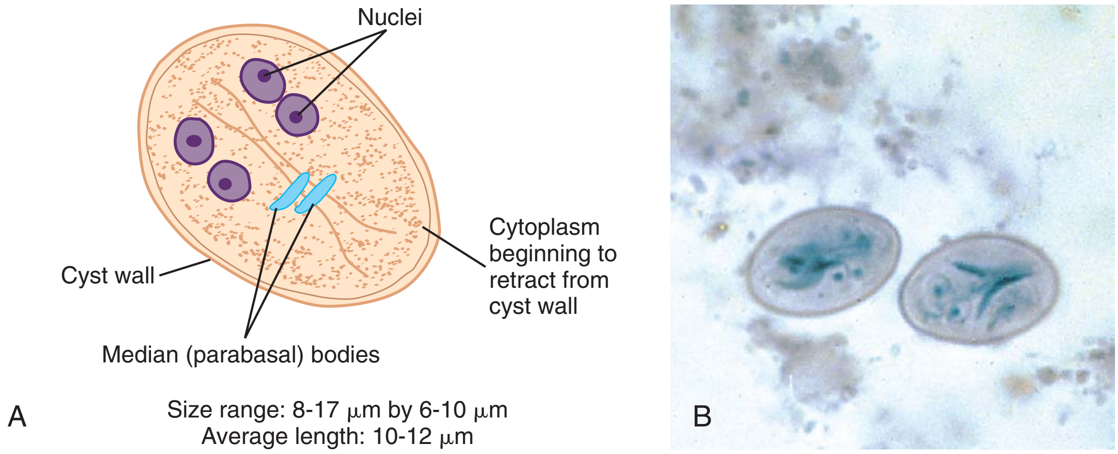


FIGURE 4-4 **A,** *Giardia intestinalis* cyst. **B,** *Giardia intestinalis* cyst. (*B from Forbes BA, Sahm DF, Weissfeld AS: Bailey & Scott's diagnostic microbiology, ed 12, St Louis, 2007, Mosby.*)

usually centrally located. Peripheral chromatin is absent. These nuclei are best detected on permanently stained specimens. The trophozoite is supported by an axostyle made up of two **axonemes,** defined as the interior portions of the flagella. Two slightly curved rodlike structures, known as **median bodies,** sit on the axonemes posterior to the nuclei.

It is important to note that there is some confusion regarding the proper name of the median bodies. Some texts refer to these structures as parabasal bodies rather than median bodies, suggesting that the two structures are different. Other texts consider median bodies and parabasal bodies as two names for the same structure. For the purposes of this text, the term *median body* is used to define structures believed to be associated with energy, metabolism, or support. Their exact function is unclear. Although they are sometimes difficult to detect, the typical *G. intestinalis* trophozoite has four pairs of flagella. One pair of flagella originates from the anterior end and one pair extends from the posterior end. The remaining two pairs of flagella are located laterally, extending from the axonemes in the center of the body. The *G. intestinalis* trophozoite is equipped with a sucking disc. Covering 50% to 75% of the ventral surface, the sucking disk serves as the nourishment point of entry by attaching to the intestinal villi of an infected human.

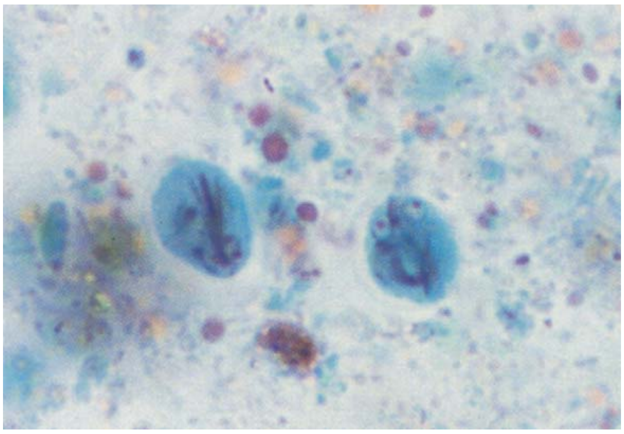


FIGURE 4-5 *Giardia intestinalis* cyst. Note red-staining nuclei (trichrome stain, ×1000).

Cysts. The typical ovoid *G. intestinalis* cyst ranges in size from 8 to 17 µm long by 6 to 10 µm wide, with an average length of 10 to 12 µm (Figs. 4-4 and 4-5; Table 4-2). The colorless and smooth cyst wall is prominent and distinct from the interior of the organism. The cytoplasm is often retracted away from the cyst wall, creating a clearing zone. This phenomenon is especially possible after being preserved in formalin. The immature cyst contains two nuclei and two median bodies. Four nuclei, which may be seen in iodine wet preparations as well as on permanent stains, and four median bodies are present in the fully mature cysts. Mature

TABLE 4-2 *Giardia intestinalis* Cyst: Typical Characteristics at a Glance

Parameter	Description
Size range	8-17 µm long 6-10 µm wide
Shape	Ovoid
Nuclei	Immature cyst, two Mature cyst, four Central karyosomes No peripheral chromatin
Cytoplasm	Retracted from cell wall
Other structures	Median bodies: two in immature cyst or four in fully mature cyst Interior flagellar structures*

*Twice as many in mature cyst as compared with immature cyst.

cysts contain twice as many interior flagellar structures.

Laboratory Diagnosis

The specimen of choice for the traditional recovery technique of *G. intestinalis* trophozoites and cysts is stool. It is important to note that *Giardia* is often shed in the stool in showers, meaning that many organisms may be passed and recovered on one day's sample and the following day's sample may reveal no parasites at all. Thus, examination of multiple samples is recommended prior to reporting that a patient is free of *Giardia*. Duodenal contents obtained by aspiration, as well as upper small intestine biopsies, may also be collected for examination. Duodenal contents can identify *G. intestinalis* using the string test, also known as Enterotest.

Several other diagnostic techniques are available for identifying *G. intestinalis,* including fecal antigen detection by enzyme immunoassays (EIA) and enzyme-linked immunosorbent assay (ELISA). Direct Fluorescence detection of both *Giardia* and *Cryptosporidium* (see Chapter 7), as well as a *Giardia* Western immunoblotting (blot) test have shown promising results in recent studies.

The newest form of identifying *Giardia* is using real-time polymerase chain reaction (RT-PCR). This molecular method is sensitive enough for environment monitoring because studies suggest that a single *Giardia* cyst may be detected using molecular methods.

Life Cycle Notes

On ingestion, the infective *G. intestinalis* cysts enter the stomach. The digestive juices, particularly gastric acid, stimulate the cysts to excyst in the duodenum. The resulting trophozoites become established and multiply approximately every 8 hours via longitudinal binary fission. The trophozoites feed by attaching their sucking disks to the mucosa of the duodenum. Trophozoites may also infect the common bile duct and gallbladder. Changes that result in an unacceptable environment for trophozoite multiplication stimulate encystation, which occurs as the trophozoites migrate into the large bowel. The cysts enter the outside environment via the feces and may remain viable for as long as 3 months in water. Trophozoites entering into the outside environment quickly disintegrate.

Epidemiology

G. intestinalis may be found worldwide—in lakes, streams, and other water sources—and are considered to be one of the most common intestinal parasites, especially among children. Ingestion of water contaminated with *G. intestinalis* is considered to be the major cause of parasitic diarrheal outbreaks in the United States. It is interesting to note that *G. intestinalis* cysts are resistant to the routine chlorination procedures carried out at most water plant facilities. Filtration as well as chemical treatment of this water is crucial to obtain adequate drinking water. In addition to contaminated water, *G. intestinalis* may be transmitted by eating contaminated fruits or vegetables. Person-to-person contact through oral-anal sexual practices or via the fecal-oral route may also transfer *G. intestinalis*.

There are a number of groups of individuals at a high risk of contracting *G. intestinalis*, including children in day care centers, people living in poor sanitary conditions, those who travel to and drink contaminated water in known endemic areas, and those who practice unprotected sex, particularly homosexual males. There are several known animal reservoir hosts, including beavers, muskrats, and water voles. In addition, there is evidence to suggest that domestic sheep, cattle, and dogs may also harbor the parasite, and perhaps may even transmit the parasite directly to humans.

Clinical Symptoms

G. intestinalis was for many years considered to be a nonpathogen. This organism is now considered to be the only known pathogenic intestinal flagellate.

Asymptomatic Carrier State. Infections with *G. intestinalis* are often completely asymptomatic.

Giardiasis (Traveler's Diarrhea). Symptomatic infections with *Giardia* may be characterized by a wide variety of clinical symptoms, ranging from mild diarrhea, abdominal cramps, anorexia, and flatulence to tenderness of the epigastric region, steatorrhea, and malabsorption syndrome. Patients suffering from a severe case of giardiasis produce light-colored stools with a high fat content that may be caused by secretions produced by the irritated mucosal lining. Fat-soluble vitamin deficiencies, folic acid deficiencies, hypoproteinemia with hypogammaglobulinemia, and structural changes of the intestinal villi may also be observed in these cases. It is interesting to note that blood rarely, if ever, accompanies the stool in these patients.

The typical incubation period for *G. intestinalis* is 10 to 36 days, after which symptomatic patients suddenly develop watery, foul-smelling diarrhea, steatorrhea, flatulence, and abdominal cramping. In general, *Giardia* is a self-limiting condition that typically is over in 10 to 14 days after onset. In chronic cases, however, multiple relapses may occur. Patients with intestinal diverticuli or an immunoglobulin A (IgA) deficiency appear to be particularly susceptible to reoccurring infections. It has been suggested that hypogammaglobulinemia may predispose to *Giardia* as well as achlorhydria. An in-depth study of the immunologic and chemical mechanics behind these suggestions, as well as other possible immunologic roles in giardiasis, is beyond the scope of this chapter.

Treatment

The primary choice of treatments for *G. intestinalis* infections, according to the Centers for Disease Control and Prevention (CDC), are metronidazole (Flagyl), tinidazole (Tindamax) and nitazoxanide (Alinia). According to the Food and Drug Administration (FDA) metronidazole, however, is not approved for *G. intestinalis* infections due to a proven increased incidence of carcinogenicity in mice and rats. Tinidazole is approved by the FDA for *G. intestinalis* infections, but is potentially carcinogenic in rats and mice due to the similar structure and biologic effects to that of metronidazole. Tinidazole is as effective as metronidazole and shows to be well tolerated in patients. Nitazoxanide is very efficient in treating adults and children and is similar in use to metronidazole, but is approved by the FDA for the treatment of diarrhea related to *Giardia* infections.

Prevention and Control

The steps necessary to prevent and control *G. intestinalis* are similar to those for *Entamoeba histolytica*. Proper water treatment that includes a combination of chemical therapy and filtration, guarding water supplies against contamination by potential reservoir hosts, exercising good personal hygiene, proper cleaning and cooking of food, and avoidance of unprotected oral-anal sex are among the most important steps to prevent and control *G. intestinalis*. Campers and hikers are encouraged to be equipped with bottled water. Double-strength saturated iodine solution may be added to potentially contaminated water prior to consuming. Portable water purification systems are also available and appear to be effective. It is imperative that individuals follow the manufacturer's directions when treating water with iodine or when using the purification system to ensure the safest drinking water possible.

Notes of Interest and New Trends

Giardia intestinalis was discovered in 1681 by Anton van Leeuwenhoek when he examined a sample of his own stool. The first known rough description of *Giardia* was, however, written later by the Secretary of the Royal Society of London, Robert Hooke.

The first recorded water outbreak of *G. intestinalis* occurred in St. Petersburg, Russia, and involved a group of visiting travelers. *Giardia* was also recognized during World War I as being responsible for diarrheal epidemics that occurred among the fighting soldiers. Increased travel in the 1970s allowed for Americans traveling to the former Soviet Union to become infected with *Giardia*. Between 1965 and 1984, over 90 water outbreaks (occurring in town and city public water supplies) were recorded in the United States.

There are several documented reports suggesting that a marked increase in the prevalence of *G. intestinalis* has occurred in the male homosexual population in recent years.

A series of two studies on the prevalence of parasites in the St. Louis area from 1988 through 1993 concluded that *G. intestinalis* was the most common parasite reported. It is interesting to note that in both studies accurate epidemiologic information regarding parasite prevalence was difficult to obtain, partly because many parasitic infections are never reported to the proper authorities.

Giardia trophozoites have often been referred to as resembling an old man with whiskers, a cartoon character, and/or a monkey's face.

A number of studies have suggested that several zymodemes of *G. intestinalis* exist. This may prove to be valuable information in the future as more so-called secrets about *Giardia* are revealed.

G. intestinalis and *E. histolytica* cysts, as well as a host of other parasites, were isolated in samples acquired from the Hudson River and East River in New York City in the early 1980s. Almost 25% of scuba divers in the New York City police and fire departments, who have been known to dive in these waters, tested positive for both parasites.

G. intestinalis and *Trichomonas vaginalis* (see later) are both known to be carriers of double-stranded RNA viruses.

Quick Quiz! 4-4

The proposed function(s) of the median bodies seen in *G. intestinalis* is (are) which of the following? (Objective 4-9 B)

A. Support
B. Energy
C. Metabolism
D. All of the above

Quick Quiz! 4-5

Which specimen type and collection regimen would be most appropriate for the diagnosis of *G. intestinalis?* (Objective 4-8)

A. One stool sample
B. Two stool samples
C. Multiple stool samples collected on subsequent days
D. One stool sample and one blood sample

Quick Quiz! 4-6

G. intestinalis trophozoites attach to the mucosa of the duodenum and feed with the assistance of this morphologic structure. (Objective 4-9B)

A. Sucking disk
B. Axostyle
C. Axoneme
D. Nucleus

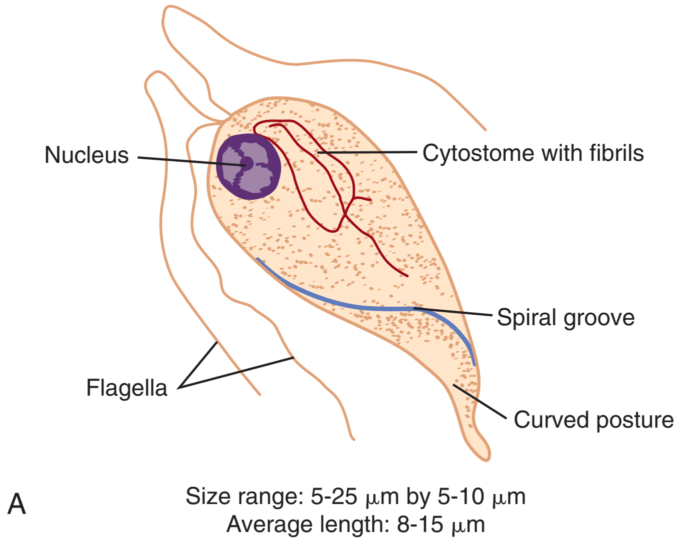


FIGURE 4-6 **A,** *Chilomastix mesnili* trophozoite. **B,** *Chilomastix mesnili* trophozoite. *(**B** from Forbes BA, Sahm DF, Weissfeld AS: Bailey & Scott's diagnostic microbiology, ed 12, St Louis, 2007, Mosby.)*

Quick Quiz! 4-7

Individuals become infected with *G. intestinalis* by which of the following? (Objective 4-5C)

A. Swimming in contaminated water
B. Ingesting contaminated food or drink
C. Inhalation of infective cysts
D. Walking barefoot on contaminated soil

Quick Quiz! 4-8

Individuals at risk for contracting *G. intestinalis* when camping and hiking are encouraged to take which of these steps to prevent infection? (Objective 4-7C)

A. Treat potentially infected water with a double-strength saturated saline solution prior to consuming.
B. Use only bottled water for drinking, cooking & appropriate personal hygiene.
C. Avoid swimming in contaminated water.
D. Wear shoes at all times.

Chilomastix mesnili
(ki″lo-mas′tiks/mes′nil′i)

Common associated disease and condition names: None (considered a nonpathogen).

TABLE 4-3 ***Chilomastix mesnili* Trophozoite: Typical Characteristics at a Glance**

Parameter	Description
Size range	5-25 μm long 5-10 μm wide
Shape	Pear-shaped
Motility	Stiff, rotary, directional
Nuclei	One with small central or eccentric karyosome No peripheral chromatin
Flagella	Four: Three extending from anterior end One extending posteriorly from cytostome region
Other structures	Prominent cytostome extending 1/3 to 1/2 body length Spiral groove

Morphology

Trophozoites. The pear-shaped *Chilomastix mesnili* trophozoite ranges from 5 to 25 μm long by 5 to 10 μm wide, with an average length of 8 to 15 μm (Fig. 4-6; Table 4-3). The broad anterior end tapers toward the posterior end of the organism. Stiff rotary motility in a directional

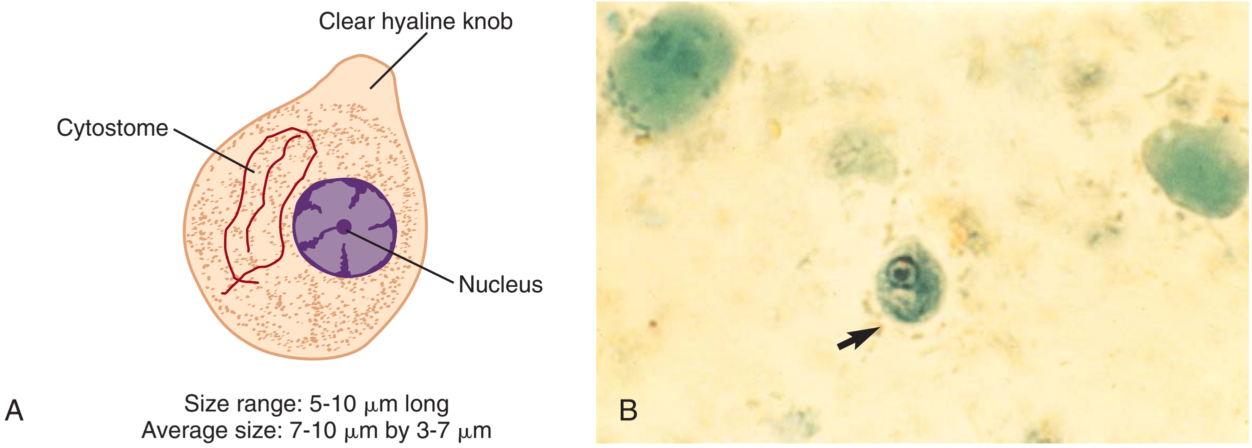


FIGURE 4-7 **A,** *Chilomastix mesnili cyst.* **B,** *Chilomastix mesnili cyst. (**B** from Mahon CR, Lehman DC, Manuselis G: Textbook of diagnostic microbiology, ed 4, St Louis, 2011, Saunders.)*

pattern is typical of the *C. mesnili* trophozoite. The single nucleus, which is usually not visible in unstained preparations, is located in the anterior end of the trophozoite. The typical small karyosome may be found located centrally or eccentrically in the form of chromatin granules that form plaques against the nuclear membrane. Peripheral chromatin is absent. *C. mesnili* trophozoites characteristically have four flagella. Three of the flagella, which seldom stain, extend out of the anterior end of the organism. The fourth flagellum is shorter than the others and extends posteriorly from a rudimentary mouth referred to as a **cytostome**. Extending one third to one half of the body length, the cytostome is prominently located to one side of the nucleus. The structure bordering the cytostome resembles a shepherd's crook and is the most prominent of several supporting cytostomal fibrils found in this area. The ventral surface indentation located toward the center of the body that extends down toward the posterior end of the trophozoite is known as a typical spiral groove. The presence of this spiral groove results in a curved posture at the posterior end.

Cysts. The cysts of *C. mesnili* are usually lemon-shaped and possess a clear anterior hyaline knob. The average cyst measures 7 to 10 μm long and 3 to 7 μm in width, but may range in length from 5 to 10 μm (Fig. 4-7; Table 4-4). A large single nucleus, consisting of a large central karyosome and no peripheral chromatin, is usually located toward the anterior end of the cyst. The well-defined cytostome, with its accompanying fibrils, may be found to one side of the nucleus.

TABLE 4-4 ***Chilomastix mesnili* Cyst: Typical Characteristics at a Glance**

Parameter	Description
Size range	5-10 μm long
Shape	Lemon-shaped, with a clear hyaline knob extending from the anterior end
Nuclei	One, with large central karyosome No peripheral chromatin
Other structures	Well-defined cytostome located on one side of the nucleus

Laboratory Diagnosis

Traditional examination of freshly passed liquid stools from patients infected with *C. mesnili* typically reveals only trophozoites. Formed stool samples from these patients usually reveal only cysts. Samples of semiformed consistency may contain trophozoites and cysts. It is interesting to note that encystation has been known to occur in unformed samples, particularly during the process of centrifuging the sample. Iodine wet preparations often demonstrate the organism's features most clearly.

Epidemiology

C. mesnili is cosmopolitan in its distribution and prefers warm climates. Those in areas in which personal hygiene and poor sanitary conditions prevail are at the greatest risk of *C. mesnili* introduction. The transmission of *C. mesnili* occurs when infective cysts are ingested. This may occur primarily through hand-to-mouth contamination or via contaminated food or drink.

Clinical Symptoms

Infections with *C. mesnili* are typically asymptomatic.

Treatment

Treatment for persons infected with *C. mesnili* is usually not indicated because this organism is considered to be a nonpathogen.

Prevention and Control

Proper personal hygiene and public sanitation practices are the two primary prevention and control measures necessary to eradicate future infections with *C. mesnili*.

Quick Quiz! 4-9

Which of the following are key morphologic characteristics of *C. mesnili?* (Objective 4-9A)

A. Round and four to eight nuclei
B. Oval and presence of a cytosome
C. Round and presence of an axoneme
D. Lemon-shaped and presence of a cytosome

Quick Quiz! 4-10

A liquid stool is the specimen of choice for the recovery of which of these morphologic forms of *C. mesnili?* (Objective 4-8)

A. Trophozoites only
B. Cysts only
C. Trophozoites and cysts

Dientamoeba fragilis
(dye-en'tuh-mee'buh/fradj"i-lis)

Common associated disease and condition names: *Dientamoeba fragilis* infection (symptomatic).

Morphology

D. fragilis was initially classified as an ameba because this organism moves by means of pseudopodia and does not have external flagella. Further investigation using electron microscopy studies has suggested that *D. fragilis* does have flagellate characteristics. It is interesting to note that the specific findings of these studies are not included in a number of texts under the discussion of this organism. Some authorities classify this organism as strictly a flagellate, whereas others list it in the flagellate section but consider it in a group of its own as an ameba-flagellate. Needless to say, there appears to still be some controversy over the correct classification of *D. fragilis*. For our purposes, *D. fragilis* will be considered as a member of the flagellates.

Trophozoites. The typical *D. fragilis* trophozoite is irregular and roundish in shape and ranges in size from 5 to 18 μm, with an average size of 8 to 12 μm (Fig. 4-8; Table 4-5). The trophozoite's progressive motility, seen primarily in freshly passed stool samples, is accomplished by broad hyaline pseudopodia that possess characteristic serrated margins. The typical *D. fragilis* trophozoite has two nuclei, each consisting of four to eight centrally located massed chromatin granules that are usually arranged in a symmetrical fashion. Peripheral chromatin is absent. The nuclei are generally only observable with

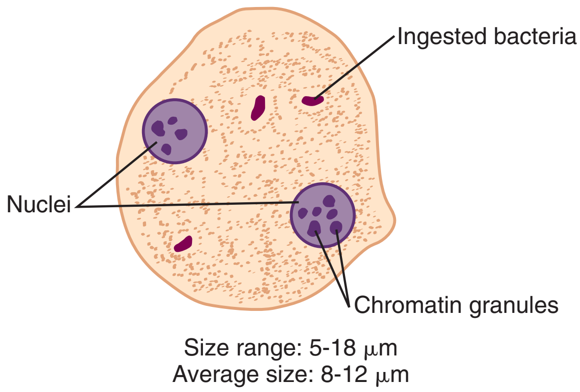


FIGURE 4-8 *Dientamoeba fragilis* trophozoite.

TABLE 4-5 *Dientamoeba fragilis* Trophozoite: Typical Characteristics at a Glance

Parameter	Description
Size range	5-18 μm
Shape	Irregularly round
Motility	Progressive, broad hyaline pseudopodia
Number of nuclei	Two, each consisting of massed clumps of four to eight chromatin granules No peripheral chromatin
Cytoplasm	Bacteria-filled vacuoles common

permanent stain. The stain of choice for distinguishing the individual chromatin granules is iron hematoxylin. Although most trophozoites are binucleated—hence, the name *Dientamoeba*—mononucleated forms may also exist. In addition, trophozoites containing three or even four nuclei may occasionally be seen. Vacuoles containing bacteria may be present in the cytoplasm of these trophozoites.

Cysts. There is no known cyst stage of *D. fragilis*.

Laboratory Diagnosis

Examination of stool samples for the presence of trophozoites is the method of choice for the laboratory diagnosis of *D. fragilis*. Multiple samples may be necessary to rule out the presence of this organism because the amount of parasite shedding may vary from day to day. In addition, it is important to note that *D. fragilis* may be difficult to find, much less identify, in typical stool samples. This organism has the ability to blend in well with the background material in the sample. In some cases, the organisms stain faintly and may not be recognized. As noted, care should be exercised when screening all unknown samples. *D. fragilis* may be missed if the sample is not properly examined.

More recently, both conventional and real-time polymerase chain reaction (RT-PCR) methods have been used to diagnose *D. fragilis* in patients. A recent study evaluated methods of detection for *D. fragilis* and RT-PCR was shown to be the most sensitive of all diagnostic methods.

Life Cycle Notes

The complete life cycle of *D. fragilis* is not well understood. Once inside the human body, however, it is known that *D. fragilis* resides in the mucosal crypts of the large intestine. There is no evidence to suggest that *D. fragilis* trophozoites invade their surrounding tissues. *D. fragilis* has only rarely been known to ingest red blood cells. Other specific information regarding the organism's life cycle remains unclear.

Epidemiology

The exact mode of *D. fragilis* transmission remains unknown. One unproven theory suggests that *D. fragilis* is transmitted via the eggs of helminth parasites such as *Enterobius vermicularis* and *Ascaris lumbricoides* (both of these organisms are discussed in detail in Chapter 8). Several studies aimed at answering this question have concluded that a notable frequency of organisms resembling *D. fragilis* were identified in patients who were also infected with *E. vermicularis* (pinworm). Data collected and studied to date indicated that this organism is most likely distributed in cosmopolitan areas. Partly because the mode of transmission remains a mystery, the specific geographic distribution of *D. fragilis* is unknown.

Demographic information collected during studies and surveys in the last 10 to 15 years has indicated that the following individuals appear to be at risk of contracting *D. fragilis:* children, homosexual men, those living in semicommunal groups, and persons who are institutionalized. These data may support the theory that *D. fragilis* transmission may occur by the fecal-oral and oral-anal routes, as well as by the person-to-person route, as the unproven theory described earlier indicates.

Other factors that may potentially inhibit accurate *D. fragilis* epidemiologic information include the fact that infection, when it occurs, is often not reported; in some cases, samples are rarely collected for study and clinicians may experience difficulty in correctly identifying the organism because of its ability to blend in with the background material of the sample.

Clinical Symptoms

Asymptomatic Carrier State. It is estimated that most people with *D. fragilis* infection remain asymptomatic.

Symptomatic. Patients who suffer symptoms associated with *D. fragilis* infections often present with diarrhea and abdominal pain. Other documented symptoms that may occur include bloody or mucoid stools, flatulence, nausea or vomiting, weight loss, and fatigue or weakness. Some patients experience diarrhea alternating with constipation, low-grade eosinophilia, and pruritus.

Treatment

Although there is some controversy over the pathogenicity of *D. fragilis,* symptomatic cases of infection may indicate treatment. The treatment of choice for such infections is iodoquinol. Tetracycline is an acceptable alternative treatment. Paromomycin (Humatin) may be used in cases when the treatments listed earlier, for whatever reason, are not appropriate.

Prevention and Control

Because so little is known about the life cycle of *D. fragilis*, especially the transmission phase, designing adequate prevention and control measures is difficult. It is believed that maintaining personal and public sanitary conditions and avoidance of unprotected homosexual practices will at least help minimize the spread of *D. fragilis* infections. If the unproven transmission theory is valid, the primary prevention and control measure would be the eradication of the helminth eggs, especially those of the pinworm.

Notes of Interest and New Trends

D. fragilis differs from the amebic trophozoites when mounted in water preparations. Although both types of organisms swell and rupture under these conditions, only *D. fragilis* returns to its normal size. Numerous granules are present in this stage and exhibit Brownian motion. This is known as the Hakansson phenomenon; it is a feature diagnostic for the identification of *D. fragilis*.

Quick Quiz! 4-11

A flagellate trophozoite that could be described as 9 to 12 µm with one or two nuclei, each with four symmetrically positioned chromatin granules and vacuoles containing bacteria in the cytoplasm, would most likely be which of the following? (Objective 4-9C)

A. *Giardia intestinalis*
B. *Dientamoeba fragilis*
C. *Chilomastix mesnilli*
D. *Blastocystis hominis*

Quick Quiz! 4-12

The permanent stain of choice for observing the nuclear features of *D. fragilis* is which of the following? (Objective 4-12)

A. Trichrome
B. Iodine
C. Saline
D. Iron hematoxylin

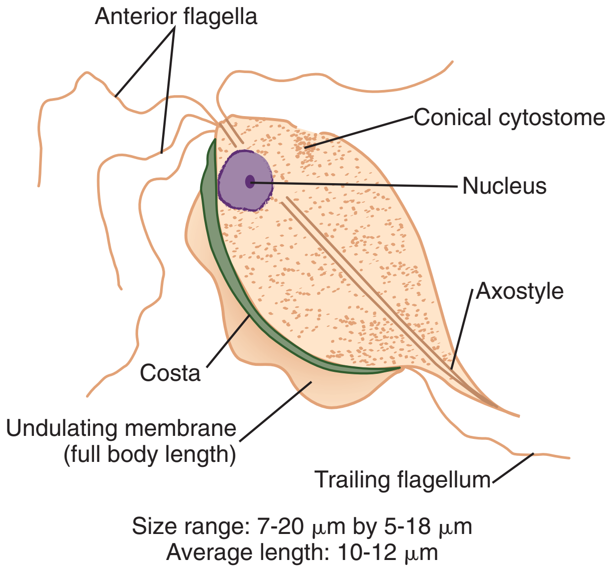


FIGURE 4-9 *Trichomonas hominis* trophozoite.

Trichomonas hominis
(trick″o-mo′nas/hŏm′ĭ-nĭs)

Common associated disease and condition names: None (considered as a nonpathogen).

Morphology

Trophozoites. Ranging in size from 7 to 20 µm long by 5 to 18 µm wide, with an average length of 10 to 12 µm, the typical *Trichomonas hominis* trophozoite is pear-shaped (Fig. 4-9; Table 4-6). The characteristic nervous, jerky motility is accomplished with the assistance of a full body-length undulating membrane. The rodlike structure located at the base of the undulating membrane, known as the **costa,** connects the undulating membrane to the trophozoite body. The single nucleus, not visible in unstained preparations, is located in the anterior region of the organism. The small central karyosome is surrounded by a delicate nuclear membrane. Peripheral chromatin is absent. The trophozoite is supported by an axostyle that extends beyond the posterior end of the body. A cone-shaped cytostome cleft may be seen in the anterior region of the organism lying ventrally opposite the undulating membrane. The typical *T. hominis* trophozoite has three to five flagella that originate from the anterior end. The single posterior flagellum is an extension of the posterior end of the undulating membrane.

Cysts. There is no known cyst form of *T. hominis*.

TABLE 4-6 ***Trichomonas hominis* Trophozoite: Typical Characteristics at a Glance**

Parameter	Description
Size range	7-20 µm long 5-18 µm wide
Shape	Pear-shaped
Motility	Nervous, jerky
Nuclei	One, with a small central karyosome No peripheral chromatin
Flagella	Three to five anterior One posterior extending from the posterior end of the undulating membrane
Other features	Axostyle that extends beyond the posterior end of the body Full body length undulating membrane Conical cytostome cleft in anterior region ventrally located opposite the undulating membrane

Laboratory Diagnosis

Stool examination is the method of choice for the recovery of *T. hominis* trophozoites.

Epidemiology

T. hominis is found worldwide, particularly in cosmopolitan areas of warm and temperate climates. It is interesting to note that the frequency of infections is higher in warm climates and that children appear to contract this parasite more often than adults. Transmission most likely occurs by ingesting trophozoites. Contaminated milk is suspected of being one of the sources of infection. It is suspected that in patients suffering from achlorhydria, the milk acts as a shield for the *T. hominis* trophozoites upon entry into the stomach. This may account for the organism's ability to survive passage through the stomach area and to settle in the small intestine. Fecal-oral transmission may also occur.

Clinical Symptoms

Infections with *T. hominis* are generally asymptomatic.

Treatment

T. hominis is considered to be a nonpathogen. Treatment, therefore, is usually not indicated.

Prevention and Control

Improved personal and public sanitary practices are crucial to the prevention and control of *T. hominis*.

Quick Quiz! 4-13

The specimen of choice for the recovery of *T. hominis* is which of the following? (Objective 4-8)

A. Stool
B. Urine
C. Intestinal contents
D. Gastric contents

Quick Quiz! 4-14

Trichomonas hominis can be transmitted by which of the following? (Objective 4-5C)

A. Contaminated milk
B. Bite of an infected mosquito
C. Ingestion of an embryonated ovum
D. Ingestion of undercooked meat

Enteromonas hominis

(ĕn′tĕr-mō′năs/hōm′ĭ-nĭs)

Common associated disease and condition names: None (considered as a nonpathogen).

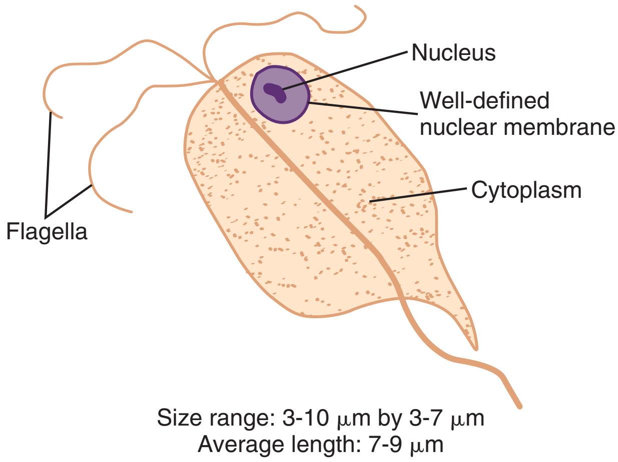


FIGURE 4-10 *Enteromonas hominis* trophozoite.

TABLE 4-7 ***Enteromonas hominis* Trophozoite: Typical Characteristics at a Glance**

Parameter	Description
Size range	3-10 μm long 3-7 μm wide
Shape	Oval; sometimes half-circle
Motility	Jerky
Nuclei	One with central karyosome No peripheral chromatin
Flagella	Four total: Three directed anteriorly One directed posteriorly
Other structures	None

Morphology

Trophozoites. *Enteromonas hominis* trophozoites typically range from 3 to 10 μm long by 3 to 7 μm wide, with an average length of 7 to 9 μm (Fig. 4-10; Table 4-7). The typical *E. hominis* trophozoite is oval in shape. This organism may also be seen in the form of a half-circle. In this case, the body is flattened on one side. *Enteromonas hominis* trophozoites usually exhibit jerky motility. The single nucleus, visible only in stained preparations, consists of a large central karyosome surrounded by a well-defined nuclear membrane. Peripheral chromatin is absent. The nucleus is located in the anterior end

TABLE 4-8	***Enteromonas hominis* Cyst: Typical Characteristics at a Glance**
Parameter	**Description**
Size range	3-10 µm long 4-7 µm wide
Shape	Oval, elongated
Nuclei	One to four Binucleated and quadrinucleated nuclei located at opposite ends Central karyosome No peripheral chromatin
Other structures	None

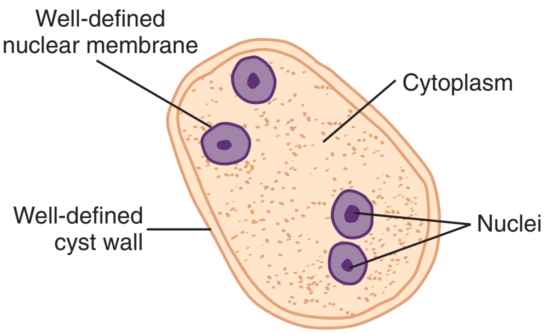


FIGURE 4-11 *Enteromonas hominis* cyst.

of the trophozoite. Four flagella originate from the organism's anterior end. Three of these flagella are directed anteriorly; the fourth is directed posteriorly. The posterior end of the organism comes together to form a structure resembling a small tail. These trophozoites are simple, relatively speaking, in that structures such as an undulating membrane, costa, cytostome, and axostyle are absent.

Cysts. The typical oval to elongated *E. hominis* cyst measures 3 to 10 µm long by 4 to 7 µm wide, with an average length of 5 to 8 µm (Fig. 4-11; Table 4-8). On first inspection of these organisms, yeast cells may often be suspected. Further investigation, however, reveals one to four nuclei. When more than one nucleus is present, these structures are typically located at opposite ends of the cell. Although binucleated cysts appear to be the most commonly encountered, quadrinucleated forms may also occur. The nuclei resemble those of the trophozoites in that each consists of a well-defined nuclear membrane surrounding a central karyosome. Peripheral chromatin is again absent. The cysts of *E. hominis* are protected by a well-defined cell wall. Fibrils and internal flagellate structures are also not seen in the cyst form. It is important to note that the size range of *E. hominis* cysts overlaps that of *Endolimax nana* cysts. A high frequency of binucleated cysts seen on a stained preparation indicates probable *E. hominis* because the probability of finding binucleated *E. nana* cysts is extremely rare.

Laboratory Diagnosis

Examination of stool samples is the laboratory diagnostic technique of choice for identifying *E. hominis* trophozoites and cysts. Unfortunately, this organism is difficult to identify accurately because of its small size. Careful screening of samples is recommended to prevent missing an *E. hominis* organism.

Epidemiology

E. hominis is distributed worldwide in warm and temperate climates. Ingestion of infected cysts appears to be the primary cause of *E. hominis* transmission.

Clinical Symptoms

Infections with *E. hominis* are characteristically asymptomatic.

Treatment

E. hominis is considered to be a nonpathogen. Treatment for *E. hominis* infections is, therefore, not indicated.

Prevention and Control

The observance of proper personal hygiene and public sanitation practices will undoubtedly result in the prevention and control of future infections with *E. hominis*.

Quick Quiz! 4-15

When *E. hominis* cysts contain more than one nuclei, where do they tend to be positioned within the cytoplasm? (Objective 4-9A)

A. Center
B. Around the periphery of the organism
C. At opposite ends of the cell
D. Throughout the organism

Quick Quiz! 4-16

Treatment is always indicated for patients when *E. hominis* is present on parasite examination. (Objective 4-7B)

A. True
B. False

Retortamonas intestinalis

(rē-tort′ă-mō′năs/ĭn″-tĕs-ti′nă-lĭs)

Common associated disease and condition names: None (considered as a nonpathogen).

Morphology

Trophozoites. The body length of a typical *Retortamonas intestinalis* trophozoite measures 3 to 7 µm, with an average of 3 to 5 µm (Fig. 4-12; Table 4-9). Ranging from 5 to 6 µm in width, the ovoid trophozoite exhibits characteristic jerky motility. A single large nucleus is present in the anterior portion of the organism. The nucleus has a somewhat small and compact central karyosome. A fine and delicate ring of chromatin granules may be visible on the nuclear membrane. Opposite the nucleus in the anterior portion of the trophozoite lies a cytostome that extends approximately half of the body length.

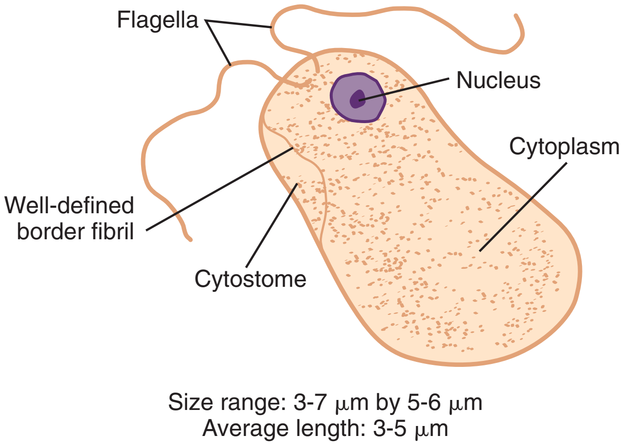


FIGURE 4-12 *Retortamonas intestinalis* trophozoite.

TABLE 4-9	*Retortamonas intestinalis* Trophozoite: Typical Characteristics at a Glance
Parameter	**Description**
Size range	3-7 µm long 5-6 µm wide
Shape	Ovoid
Motility	Jerky
Nuclei	One, with small central karyosome Ring of chromatin granules may be on nuclear membrane
Flagella	Two; anterior
Other structures	Cytostome extending halfway down body length with well-defined fibril border opposite the nucleus in the anterior end

A well-defined fibril borders this structure. The *R. intestinalis* trophozoite is equipped with only two anterior flagella.

Cysts. The lemon- to pear-shaped *R. intestinalis* cysts measure from 3 to 9 µm in length and up to 5 µm wide, with an average length of 5 to 7 µm (Fig. 4-13; Table 4-10). The single nucleus, consisting of a central karyosome, may be surrounded by a delicate ring of chromatin granules and is located in the anterior region or closer toward the center of the organism. Two fused fibrils originate anterior to the nuclear region,

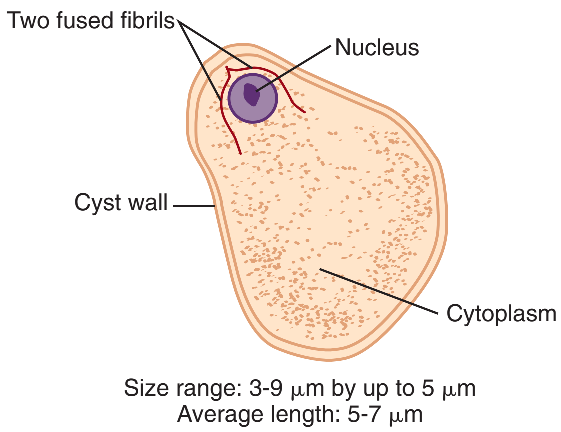


FIGURE 4-13 *Retortamonas intestinalis* cyst.

TABLE 4-10 *Retortamonas intestinalis* Cyst: Typical Characteristics at a Glance

Parameter	Description
Size range	3-9 μm long Up to 5 μm wide
Shape	Lemon-shaped, pear-shaped
Nuclei	One, located in anterior-central region with central karyosome May be surrounded by a delicate ring of chromatin granules
Other structures	Two fused fibrils resembling a bird's beak in the anterior nuclear region, only visible in stained preparations

splitting up around the nucleus, and extend separately posterior to the nucleus, forming a characteristic bird's beak. This structure, along with the nucleus itself, is often difficult to see, especially in unstained preparations.

Laboratory Diagnosis

A stained stool preparation is the best sample to examine for the presence of *R. intestinalis* trophozoites and cysts. Unfortunately, accurate identification is difficult, in part because of the small size of this organism. In addition, the small number of diagnostic features may sometimes not stain well enough to recognize. Stools suspected of containing *R. intestinalis*, as well as the other smaller flagellates, should be carefully screened before reporting a negative test result.

Epidemiology

Although *R. intestinalis* is rarely reported in clinical stool samples, its existence has been documented in warm and temperate climates throughout the world. Transmission is accomplished by ingestion of the infected cysts. A select group of individuals, including patients in psychiatric hospitals and others living in crowded conditions, have been known to contract *R. intestinalis* infections because of poor sanitation and hygiene conditions.

Clinical Symptoms

Infections with *R. intestinalis* typically do not produce symptoms.

Treatment

Because *R. intestinalis* is considered a nonpathogen, treatment is usually not indicated.

Prevention and Control

The most important *R. intestinalis* prevention and control measures are improved personal and public hygiene conditions.

Quick Quiz! 4-17

The traditional technique and specimen of choice for identifying *Retortamonas intestinalis* is which of the following? (Objectives 4-8 and 4-12)

A. Permanently stained blood
B. Iodine prep of urine
C. Saline prep of bronchial wash
D. Permanently stained stool

Quick Quiz! 4-18

Individuals contract *R. intestinalis* by which of the following? (Objective 4-5C)

A. Ingesting infective cysts in contaminated food or drink
B. Consuming trophozoites in contaminated beverages
C. Stepping barefoot on infective soil
D. Inhaling infective dust particles

Trichomonas tenax

(trick″o-mo′nas/tĕn′ăx)

Common associated disease and condition names: None (considered as a nonpathogen).

Morphology

Trophozoites. The typical *Trichomonas tenax* trophozoite is described as being oval to pear-shaped, measuring 5 to 14 µm long, with an average length of 6 to 9 µm (Fig. 4-14; Table 4-11). The single, ovoid, vesicular nucleus is filled with several chromatin granules and is usually located in the central 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. A small anterior cytostome is located next to the axostyle, opposite the undulating membrane.

Cyst. There is no known cyst stage of *T. tenax*.

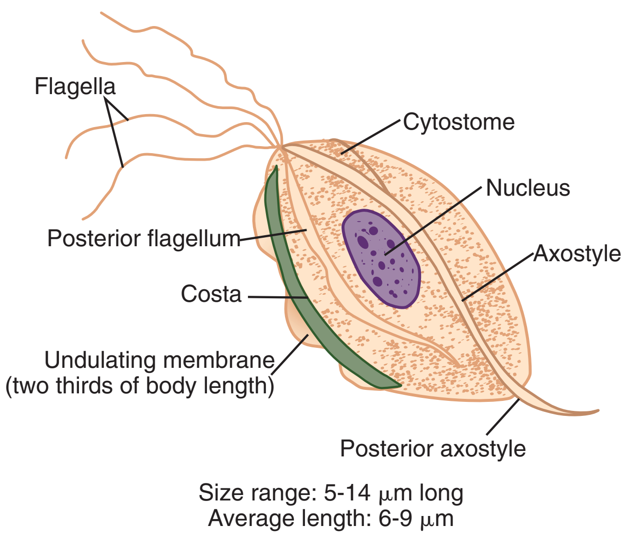


FIGURE 4-14 *Trichomonas tenax* trophozoite.

Laboratory Diagnosis

The specimen of choice for diagnosing *T. tenax* trophozoite is mouth scrapings. Microscopic examination of tonsillar crypts and pyorrheal pockets (see Chapter 2) of patients suffering from *T. tenax* infections often yields typical trophozoites. Tartar between the teeth and gingival margin of the gums are the primary areas of the mouth that may also potentially harbor this organism. Samples suspected of containing *T. tenax* may also be cultured onto appropriate media.

TABLE 4-11 ***Trichomonas tenax* Trophozoite: Typical Characteristics at a Glance**

Parameter	Description
Size range	5-14 µm long
Shape	Oval, pear-shaped
Nuclei	One, ovoid nucleus; consists of vesicular region filled with chromatin granules
Flagella	Five total, all originating anteriorly: Four extend anteriorly; One extends posteriorly
Other structures	Undulating membrane extending two thirds of body length with accompanying costa; Thick axostyle curves around nucleus; extends beyond body length; Small anterior cytostome opposite undulating membrane

However, this method is rarely used in most clinical laboratories.

Life Cycle Notes

T. tenax trophozoites survive in the body as mouth scavengers that feed primarily on local microorganisms. Located in the tartar between the teeth, tonsillar crypts, pyorrheal pockets, and gingival margin around the gums, *T. tenax* trophozoites multiply by longitudinal binary fission. These trophozoites are unable to survive the digestive process.

Epidemiology

Although the exact mode of transmitting *T. tenax* trophozoites is unknown, there is evidence suggesting that the use of contaminated dishes and utensils, as well as introducing droplet contamination through kissing, may be the routes of transmission. The trophozoites appear to be durable, surviving several hours in drinking water. Infections with *T. tenax* occur throughout the world almost exclusively in patients with poor oral hygiene.

Clinical Symptoms

The typical *T. tenax* infection does not produce any notable symptoms. On a rare occasion, *T. tenax* has been known to invade the respiratory tract, but this appears to have mainly occurred in patients with underlying thoracic or lung abscesses of pleural exudates.

Treatment

T. tenax is considered to be a nonpathogen and no chemical treatment is normally indicated. The *T. tenax* trophozoites seem to disappear in infected persons following the institution of proper oral hygiene practices.

Prevention and Control

Practicing good oral hygiene is the most effective method of preventing and controlling the future spread of *T. tenax* infections.

Quick Quiz! 4-19

How far down the body length does the *Trichomonas tenax* undulating membrane extend? (Objective 4-9A)

A. One fourth
B. One half
C. Three fourths
D. Full body

Quick Quiz! 4-20

The specimen of choice for the recovery of *Trichomonas tenax* is which of the following? (Objective 4-8)

A. Stool
B. Urine
C. Mouth scrapings
D. Cerebrospinal fluid

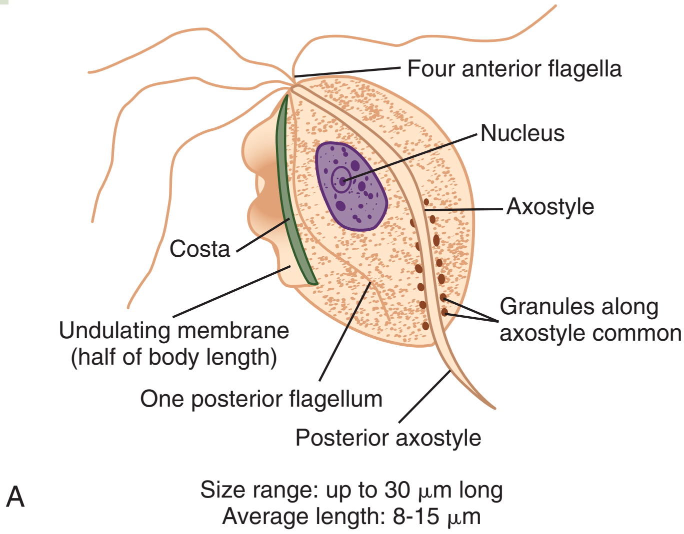
Trichomonas vaginalis

(trick″o-mo′nas/vadj-i-nay′lis)

Common associated disease and condition names: Persistent urethritis, persistent vaginitis, infant *Trichomonas vaginalis* infection.

Morphology

Trophozoites. Although typical *T. vaginalis* trophozoites may reach up to 30 µm in length, the average length is 8 to 15 µm (Fig. 4-15; Table 4-12). The trophozoites may appear ovoid, round, or pearlike in shape. Rapid jerky motility is accomplished with the aid of the organism's four to six flagella, all of which originate from the anterior end. Only one of the flagella extends posteriorly. The flagella may be difficult to find on specimen preparations. The characteristic undulating membrane is short, relatively speaking, extending only half of the body length. The single nucleus is ovoid, nondescript, and not visible in unstained preparations. *T. vaginalis* trophozoites are equipped with an easily recognizable axostyle that often curves around the nucleus and extends posteriorly beyond the body. Granules may be seen along the axostyle.



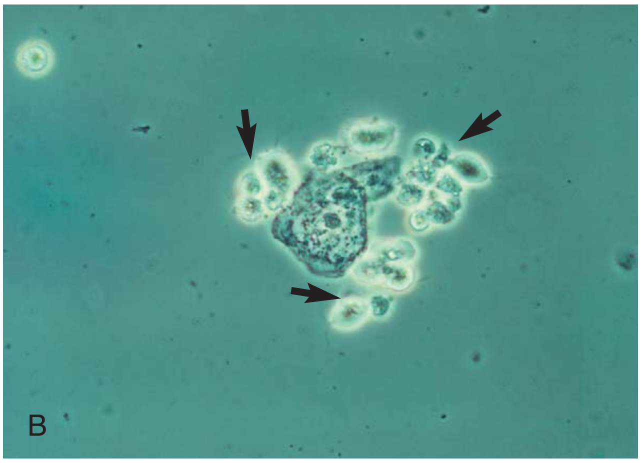


FIGURE 4-15 **A,** *Trichomonas vaginalis* trophozoite. **B,** Phase contrast wet mount micrograph of a vaginal discharge revealing the presence of *Trichomonas vaginalis* protozoa surrounding a squamous epithelial cell. *(**B** from Mahon CR, Lehman DC, Manuselis G: Textbook of diagnostic microbiology, ed 4, St Louis, 2011, Saunders; courtesy Centers for Disease Control and Prevention, Atlanta.)*

TABLE 4-12 ***Trichomonas vaginalis* Trophozoite: Typical Characteristics at a Glance**

Parameter	Description
Size range	Up to 30 µm long
Shape	Ovoid, round or pear-shaped
Motility	Rapid, jerky
Nuclei	One, ovoid, nondescript
Flagella	All originating anteriorly: Three to five extending anteriorly; One extending posteriorly
Other features	Undulating membrane extending half of body length; Prominent axostyle that often curves around nucleus; granules may be seen along axostyle

Cyst. There is no known *T. vaginalis* cyst stage.

Laboratory Diagnosis

T. vaginalis trophozoites may be recovered using standard processing methods (see Chapter 2) in spun urine, vaginal discharges, urethral discharges, and prostatic secretions. Although permanent stains may be performed, examination of saline wet preparations is preferred in many cases. Not only does the prompt examination of saline wet preparations allow the practitioner to observe the organism's active motility readily, as well as the other typical characteristics, the testing may be performed in a relatively short amount of time. Additional diagnostic tests available include phase contrast microscopy, Papanicolaou (Pap) smears, fluorescent stains, monoclonal antibody assays, enzyme immunoassays, and cultures.

A DNA-based assay has been developed for *T. vaginalis* detection using Affirm VPIII (BD Diagnostics, Sparks, MD). The sensitivity and specificity of this method of testing is much greater than with standard processing methods.

Another diagnostic tool used by laboratories today is InPouch TV (BioMed Diagnostics, White City, OR) culture system. This method can be used with vaginal swabs from women, urethral swabs from men, urine sediment and semen sediment. This method requires incubation time and takes up to 3 days before a result is determined.

Life Cycle Notes

T. vaginalis trophozoites reside on the mucosal surface of the vagina in infected women. The growing trophozoites multiply by longitudinal binary fission and feed on local bacteria and leukocytes. *T. vaginalis* trophozoites thrive in a slightly alkaline or slightly acidic pH environment, such as that commonly seen in an unhealthy vagina. The most common infection site of *T. vaginalis* in males is the prostate gland region and the epithelium of the urethra. The detailed life cycle in the male host is unknown.

Epidemiology

Infections with *T. vaginalis* occur worldwide. The primary mode of transmission of the *T. vaginalis* trophozoites is sexual intercourse. These trophozoites may also migrate through a mother's birth canal and infect the unborn child. Under optimal conditions, *T. vaginalis* is known to be transferred via contaminated toilet articles or underclothing. However, this mode of transmission is rare. The sharing of douche supplies, as well as communal bathing, are also potential routes of infection. *T. vaginalis* trophozoites, which are by nature hardy and resistant to changes in their environment, have been known to survive in urine, on wet sponges, and on damp towels for several hours, as well as in water for up to 40 minutes.

Clinical Symptoms

Asymptomatic Carrier State. Asymptomatic cases of *T. vaginalis* most frequently occur in men.

Persistent Urethritis. Persistent or recurring urethritis is the condition that symptomatic men experience as a result of a *T. vaginalis* infection. Involvement of the seminal vesicles, higher parts of the urogenital tract, and prostate may occur in severe cases of infection. Symptoms of severe infection include an enlarged tender prostate, dysuria, nocturia, and epididymitis. These patients often release a thin, white urethral discharge that contains the *T. vaginalis* trophozoites.

Persistent Vaginitis. Persistent vaginitis, found in infected women, is characterized by a foul-smelling, greenish-yellow liquid vaginal discharge after an incubation period of 4 to 28 days. Vaginal acidity present during and immediately following menstruation most likely accounts for the exacerbation of symptoms. Burning, itching, and chafing may also be present. Red punctate lesions may be present upon examining the vaginal mucosa of infected women. Urethral involvement, dysuria, and increased frequency of urination are among the most commonly experienced symptoms. Cystitis is less commonly observed but may occur.

Infant Infections. *T. vaginalis* has been recovered from infants suffering from both respiratory infection and conjunctivitis. These conditions were most likely contracted as a result of *T. vaginalis* trophozoites migrating from an infected mother to the infant through the birth canal and/or during vaginal delivery.

Treatment

With few exceptions, the treatment of choice for *T. vaginalis* infections is metronidazole (Flagyl). Because this parasite is sexually transmitted, treatment of all sexual partners is recommended.

Prevention and Control

The primary step necessary to prevent and control *T. vaginalis* infections is the avoidance of unprotected sex. In addition, the prompt diagnosis and treatment of asymptomatic men is also essential. Although the risk of contracting *T. vaginalis* by these means is relatively low, the avoidance of sharing douche equipment and communal bathing, as well as close contact with potentially infective underclothing, toilet articles, damp towels, and wet sponges, is recommended.