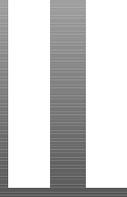
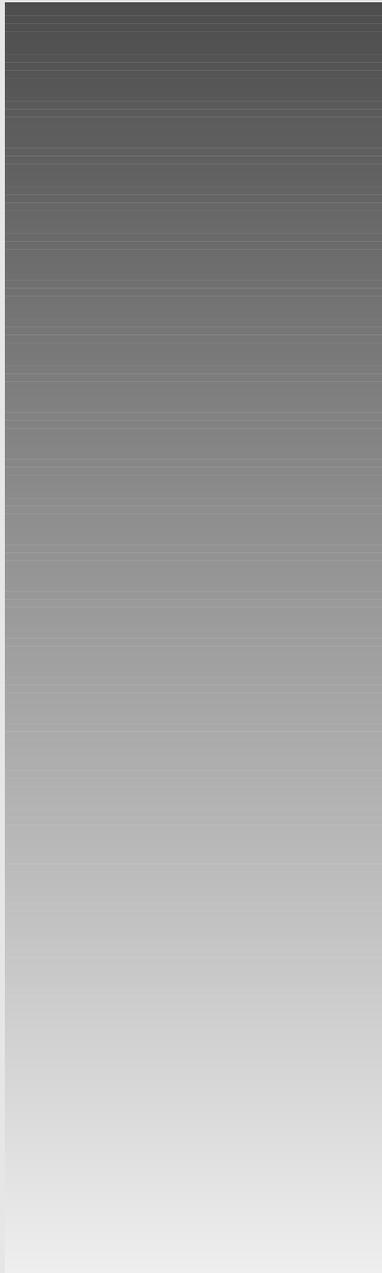


PART



Protozoa



CHAPTER FOUR

Intestinal Amebae

OUTLINE

INTESTINAL AMEBAE

Entamoeba histolytica/Entamoeba dispar
(EN-ta-MEE-buh HIS-toe-LIT-I-ka/EN-ta-MEE-buh DIS-par)

Entamoeba hartmanni
(EN-ta-MEE-buh hart-MAN-ee)

Entamoeba coli (EN-ta-MEE-buh KO-lie)

Endolimax nana (EN-do-LIE-max NAY-na)

Iodamoeba butschlii
(i-O-da-MEE-buh BOOSH-lee-i)

Blastocystis hominis (BLAS-toe-SIS-tis HOM-in-is)

LEARNING OBJECTIVES

After reading and studying this chapter, the student should be able to:

- List the clinically significant intestinal amebae found in humans.
- List and describe characteristics used to identify intestinal amebae.
- Describe the typical life cycle of intestinal amebae and note the species that may also exhibit extra-intestinal development.
- Compare the morphological characteristics of intestinal amebic trophozoites, including size, descriptions of cytoplasm, karyosomes, inclusions, and numbers and characteristics of nuclei.
- Compare the morphological characteristics of intestinal amebic cysts, including size, descriptions of cytoplasm, karyosomes, inclusions, and numbers and characteristics of nuclei.
- Describe the pathogenesis of intestinal amebiasis in humans.
- Describe the treatment of infection with pathogenic amebae.
- Describe the prevention of infection with pathogenic amebae.

KEY TERMS

Binary fission
(BI-na-ree FI-shun)

Central body
(SEN-trul BOD-ee)

Chromatin (KRO-ma-tin)

Chromatoidal bar
(KRO-ma-TOY-dal bar)

Commensals (ko-MEN-sals)

Cyst (SIST)

Dysentery (DIS-in-te-ree)

Excystation (EGG-sis-TA-shun)

Glycogen mass
(GLI-ko-jen mass)

Karyosome (KAR-ee-o-som)

Pathogen (PATH-o-jen)

Pseudopod (SOO-do-pod)

Trophozoite (TRO-fo-ZO-ite)

INTRODUCTION

Human infection with amebae is known as amebiasis. The amebae are classified in the subphylum Sarcodina, in the phylum Sarcomastigophora. These parasites are characterized by the presence of **pseudopods** (extensions of the cytoplasm, which act as organelles of locomotion). All amebae, except one, exist in two forms. These forms are the **trophozoite** (the motile, actively feeding, multiplying form, susceptible to destruction outside the host), and the **cyst** (the dormant, non-feeding resistant stage, which is significant in the transmission of amebae from host to host). Amebae multiply by **binary fission** (splitting of the parent cell, after duplication of cytoplasm and genetic material, into two equal cells).

The diagnosis of amebiasis is made in the laboratory by the examination of stool specimens for the presence of trophozoites and cysts. Trophozoites are more likely to be found in liquid stool specimens, while cysts are generally more numerous in formed stools. Important characteristics used to identify amebae include the number and structure of nuclei, especially

the presence and location of **chromatin** (genetic material found in the nucleus), particularly along the inner nuclear membrane, (peripheral nuclear chromatin). Most amebic trophozoites have a single nucleus, while the cysts are usually multinucleate. The number of nuclei is characteristic for each species of amebae. All intestinal amebae have **karyosomes** (clumps of chromatin material found within the nuclei). The size, configuration and location of the karyosomes are distinctive for each ameba and are helpful in identification.

The trophozoite cytoplasm may be finely or coarsely granular. It may be vacuolated, and may have a variety of inclusions, such as red blood cells, bacteria, or yeasts. The cyst cytoplasm is usually finely granular. Inclusions may be found in the cytoplasm. **Glycogen masses** (masses of food stored as glycogen) may be present in the cysts of certain species of amebae. **Chromatoidal bars** (rod-shaped masses of RNA) are also characteristically found. These bodies may be rodlike, with rounded, or splinter-like ends.

The characteristic features of the trophozoites and cysts of intestinal amebae are demonstrated in Figure 4-1.

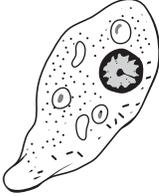
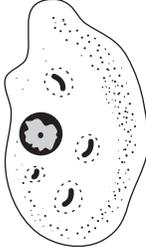
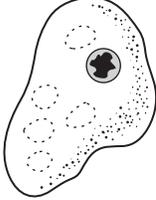
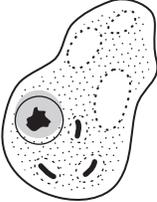
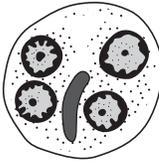
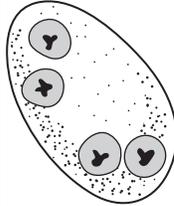
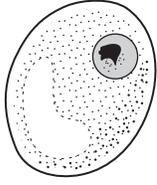
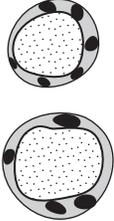
Intestinal Amebae						
	<i>Entamoeba histolytica</i>	<i>Entamoeba hartmanni</i>	<i>Entamoeba coli</i>	<i>Endolimax nana</i>	<i>Iodamoeba butschlii</i>	<i>Blastocystis hominis</i>
Trophozoite						No Trophozoite stage
Cyst						

FIG 4-1 Characteristic Features of Trophozoites and Cysts of Intestinal Amebae

INTESTINAL AMEBAE

The intestinal amebae may be recognized and identified by observation of the appearance of trophozoites and cysts in stained and unstained preparations. The only truly significant **pathogen** (disease-causing microbe) among the amebae is *Entamoeba histolytica*. This pathogen causes amebic **dysentery** (a gastrointestinal illness characterized by diarrhea, with blood and mucus in the stools). With the exception of *Blastocystis hominis*, the remaining amebae discussed in this chapter are considered to be **commensals**, which are not known to cause human illness. Their importance lies in the need to distinguish them from *E. histolytica*, as well as to provide evidence of exposure to fecally-contaminated food or water.

Entamoeba histolytica/ *Entamoeba dispar*

Morphology

Trophozoite: *Entamoeba histolytica* is the most pathogenic of the intestinal amebae found in humans, although morphologically indistinguishable nonpathogenic strains of *E. dispar* exist. The trophozoites of *E. histolytica* measure 10–60 micrometers (μm) (Figure 4–2). Trophozoite cytoplasm is finely granular, and may contain inclusions. Ingested bacteria and yeast are usually absent, although nonpathogenic strains, which tend to be somewhat smaller, may contain bacteria. The presence of red blood cells is diagnostic for *E. histolytica*. The single nucleus has peripheral nuclear chromatin, which is usually evenly distributed in a beadlike arrangement, with a small, central, compact karyosome. Movement is progressive and purposeful, and is characterized by protrusion of a clear pseudopod.

Cyst: The spherical cyst of *E. histolytica* measures 10–20 μm (Figure 4–3). The mature cyst contains 4 nuclei, while the immature cyst may contain fewer than 4. It is imperative to carefully focus up and down using the microscope, to accurately count the nuclei at different planes. The presence of evenly distributed peripheral nuclear chromatin is important in the identification of *E. histolytica*. All species of the genus *Entamoeba*, but no other genus of amebae, possess peripheral nuclear chromatin in different arrangements.

The karyosome is tiny and usually centrally located with evenly distributed peripheral nuclear chromatin. The elongated chromatoidal bars have blunt, smooth, rounded ends. Glycogen vacuoles may be present in immature cysts, but usually disappear as cysts mature.

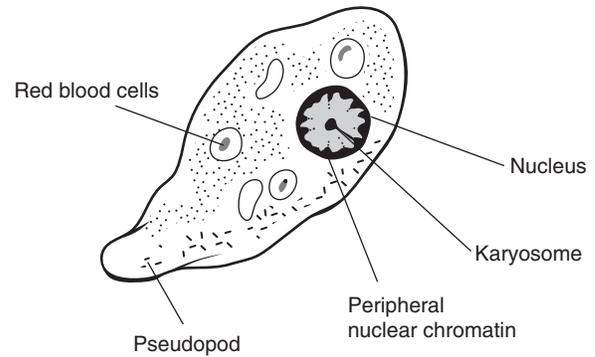


FIG 4–2 *E. histolytica* trophozoite

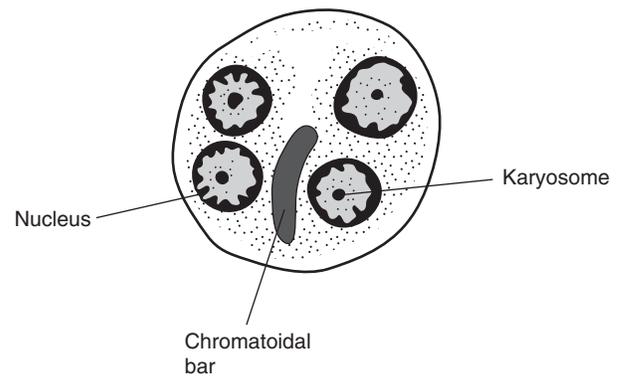


FIG 4–3 *E. histolytica* cyst

Life Cycle

The life cycle of all amebae is similar, and is shown in Figure 4–4. The invasive stage, however, involving extra-intestinal infection, is seen only with *E. histolytica*. Following ingestion, the mature cyst passes through the stomach and into the small intestine. **Excystation** occurs in the lower ileum, when the cyst develops into the trophozoite form. Binary fission follows, and trophozoites continue to multiply in the lumen of the colon. Trophozoites often become encysted. These immature cysts are passed in the feces, or develop to maturity before being excreted. Both stages of cysts plus trophozoites may be found in the feces, although trophozoites are usually only found in liquid feces. Occasionally trophozoites invade the wall of the colon, multiply, and pass into the circulation.

Transmission and Pathogenesis

E. histolytica has a wide geographic distribution and is considered one of the most important human protozoan parasites. It is now recognized that clinical isolates of *E. histolytica* may be divided into pathogenic and nonpathogenic strains (zymodemes), called *E. histolytica* and *E. dispar*, respectively. For the purposes

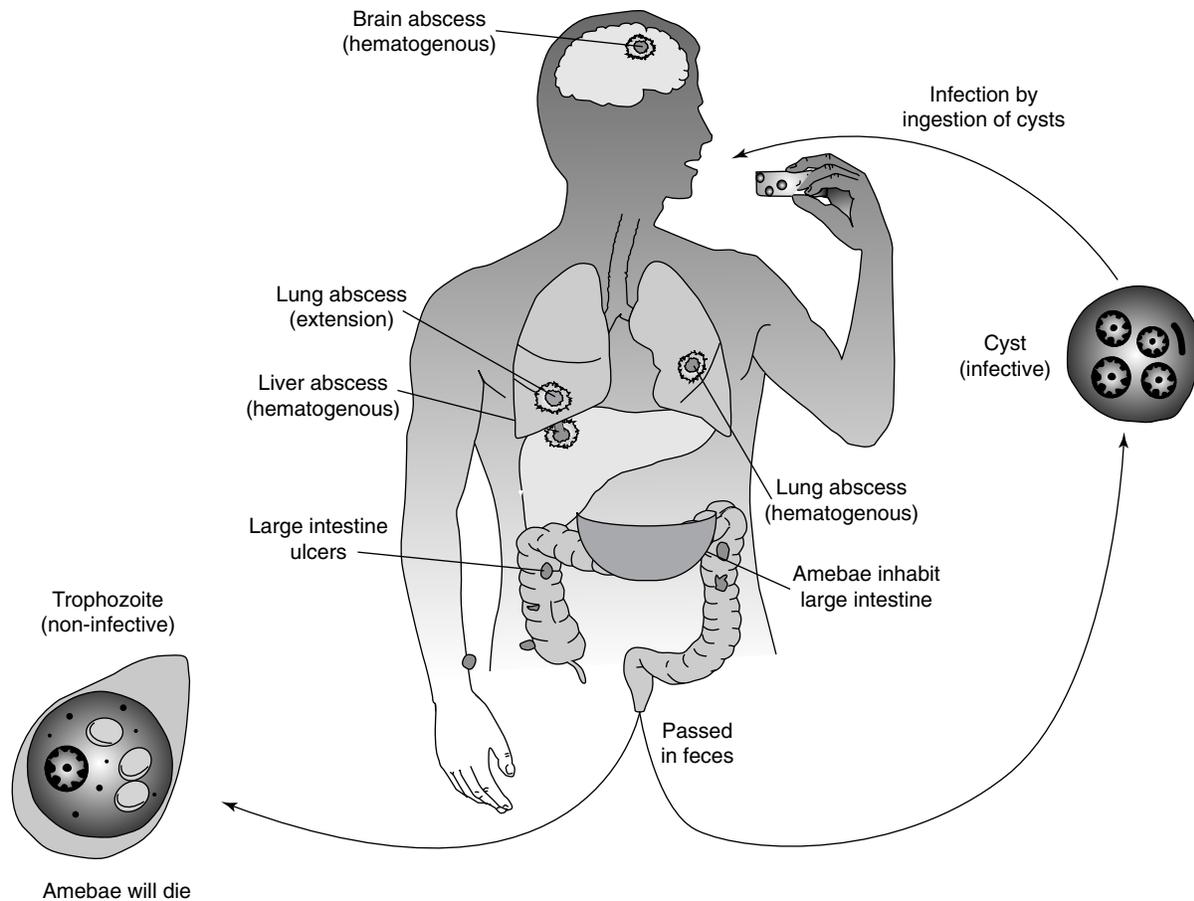


FIG 4-4 Life cycle of *Entamoeba histolytica*.

of this text, we will confine our discussion to *E. histolytica*.

Amebiasis is usually acquired by ingestion of contaminated water or food containing amebic cysts, although transmission as a sexually transmitted disease by male homosexuals has occurred. Asymptomatic carriers may also transmit disease. Incubation is variable, from several days to months. Symptomatic patients may have abdominal pain and diarrhea. When dysentery develops, bloody diarrhea may ensue. Ulcers may form in the appendix, cecum, and other parts of the colon. The characteristic flask-shaped ulcer appears to be raised, with a small mucosal opening, and an eroded area beneath the surface. Symptoms may resemble ulcerative colitis or diverticulitis. *E. histolytica* trophozoites may invade the wall of the colon, enter the circulation, and spread hematogenously, causing the development of extra-intestinal abscesses. Multiple amebic abscesses may develop, especially in the liver, but also in the brain or lung.

Laboratory Diagnosis

Differentiation among intestinal amebae is based on morphologic examination of fecal preparations for the presence of parasites. Actively motile amebic trophozoites are commonly seen in direct wet mount preparations made from liquid or soft fecal specimens from infected patients. However the preferred method involves concentration techniques, and particularly, permanent smears stained by the trichrome method. This procedure and others are described in Chapter 2. Other specimens suitable for examination include sigmoidoscopic aspirates and aspirates obtained from liver abscesses. Although trophozoites in fresh specimens seen on wet mount are usually ameboid in shape, fixation frequently causes these structures to round up or become elongated.

Serological procedures, including the indirect hemagglutination assay (IHA), the enzyme-linked immunosorbent assay (ELISA), and the indirect immunofluorescent (IFA) assay are available. These

tests are particularly useful in the diagnosis of extra-intestinal infection with *E. histolytica*. Certain methods are also helpful in monitoring the patient's therapeutic response. In addition to antibody detection, commercial kits may be used to detect parasite antigens in stool specimens. Special techniques, such as enzyme electrophoresis or DNA probes may be used to distinguish the pathogenic *E. histolytica* from the non-pathogenic *E. dispar*.

Treatment and Prevention

The presence of *E. histolytica* requires treatment of the patient regardless of symptoms. Amebicidal agents available for the treatment of amebiasis are divided into two classes, luminal amebicides, such as iodoquinol or diloxanide furoate, and tissue amebicides, including metronidazole, chloroquine and dehydroemetine. No resistance to these agents has been reported.

The human acts as the reservoir for infection with *E. histolytica*. The cysts of this parasite are hardy and often transmitted in infected water. They are resistant to environmental effects, including chlorination. A combination of filtration and chemical treatment is required to ensure a parasite-free water supply. Close attention to maintaining sanitary conditions is essential to avoid transmission of this parasite.

Entamoeba hartmanni

Morphology

Trophozoite: *E. hartmanni* looks similar to *E. histolytica*, but is much smaller (Figure 4–5). For this reason, it was previously known as “small-race histolytica.” The trophozoite measures 5–12 μm , with a small, discrete karyosome, which may be centrally located, or which may be eccentric. When seen on a wet mount, the parasite demonstrates non-progressive motility. Although bacteria may be present, no red blood cells are ingested.

Cyst: The cyst of *E. hartmanni* measures 5–10 μm . Although the mature cyst, like *E. histolytica*, contains 4 nuclei, only 2 nuclei are frequently seen. Chromatoidal bars will usually look like those found in *E. histolytica*.

Life Cycle

The life cycle of *E. hartmanni* is similar to *E. histolytica*, but lacking the extra-intestinal phase. Following excystation in the lower ileum, binary fission occurs, and trophozoites continue to multiply in the

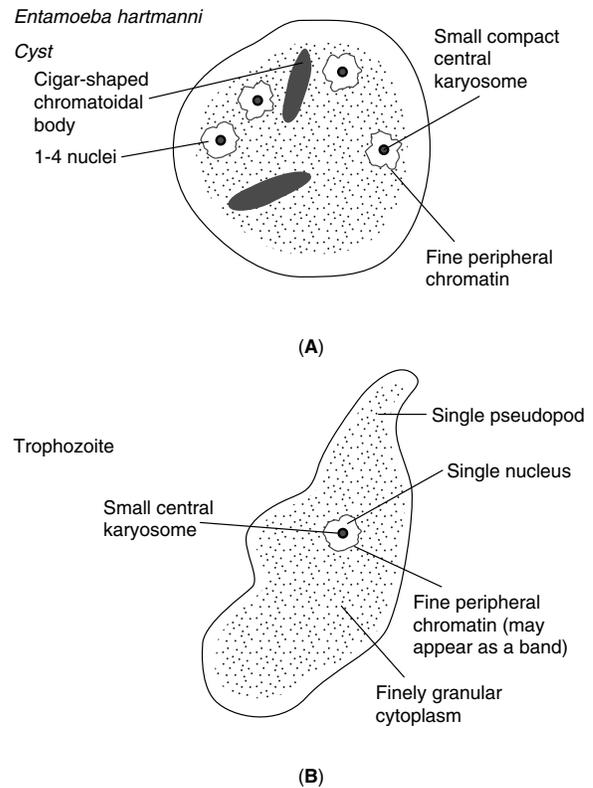


FIG 4–5 *Entamoeba hartmanni*. **A.** cyst and **B.** trophozoite.

lumen of the colon. Immature and mature cysts, as well as trophozoites, may be found in the feces.

Transmission and Pathogenesis

Amebiasis caused by *E. hartmanni* is acquired by ingestion of water or food containing mature cysts. This ameba is considered to be a nonpathogenic commensal. Infections are asymptomatic

Laboratory Diagnosis

The standard examination for ova and parasites is the most commonly used procedure to detect infection with *E. hartmanni*. Care must be taken to accurately measure the trophozoites and cysts, using a calibrated ocular micrometer, to distinguish them from the pathogenic *E. histolytica*.

Treatment and Prevention

E. hartmanni is considered nonpathogenic, therefore, no treatment is warranted. Since ingestion of contaminated water containing cysts is the usual means

of transmission, good sanitation practices should be employed to prevent infection.

Entamoeba coli

Morphology

Trophozoite: The trophozoite of *E. coli* measures 15–50 μm (Figure 4–6). The coarsely granulated cytoplasm is usually described as “dirty,” containing many vacuoles, as well as bacteria, yeast and debris. In the single nucleus, peripheral nuclear chromatin is unevenly distributed in clumps, with a large, discrete eccentric karyosome. The characteristic motility of *E. coli* is sluggish and non-progressive, with blunt pseudopods.

Cyst: The usually spherical, sometimes oval, cyst (Figure 4–7) measures 10–35 μm , and contains 8 nuclei, and as many as 16 nuclei, when mature. Immature cysts contain 8 nuclei or fewer. Peripheral chromatin is granular and unevenly distributed. Chromatoidal bars, if present, tend to have sharp, pointed, splinterlike ends.

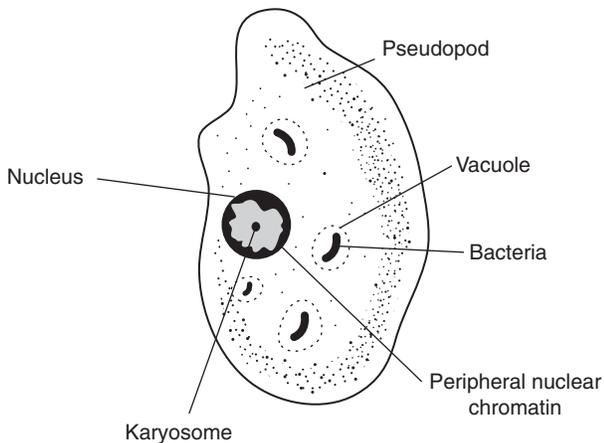


FIG 4–6 *E. coli* trophozoite

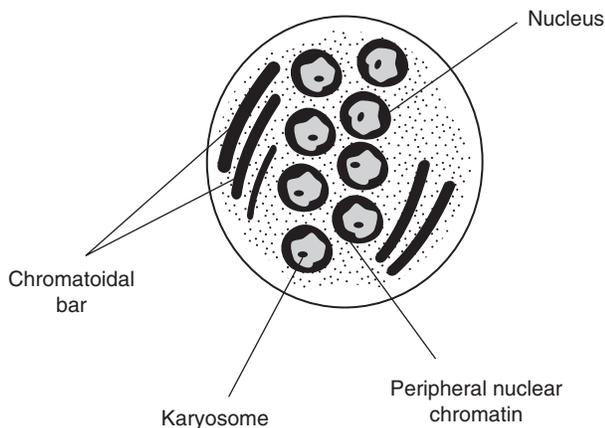


FIG 4–7 *E. coli* cyst

Life Cycle

The life cycle of *E. coli* is similar to other amebae, except for the extra-intestinal stage found in *E. histolytica*.

Transmission and Pathogenesis

Infection with *E. coli* is asymptomatic. Transmission of infection is caused by ingestion of food or water contaminated with amebic cysts. Transmission by flies and cockroaches has been reported.

Laboratory Diagnosis

Diagnosis of *E. coli* infection is made using the standard examination of fecal specimens for ova and parasites. The permanent stained smear is recommended for the detection of this parasite.

Treatment and Prevention

No treatment is recommended for the nonpathogen *E. coli*. Prevention of infection is accomplished by avoidance of contaminated food or water. Protection of food and drink from flies and cockroaches is required to stop transmission.

Endolimax nana

Morphology

Trophozoite: Trophozoites of *Endolimax nana* measure 6–12 micrometers (Figure 4–8); the granular cytoplasm is usually described as “clean” or finely vacuolated, with some nuclear variation. Bacteria may be present. No peripheral chromatin is present in the single nucleus. Normally, only karyosomes are visible, and are large and blotlike. Wet mounts reveal amebae with blunt pseudopods; motility is sluggish and non-progressive.

Cyst: The round to oval cyst (Figure 4–9) contains 4 nuclei, with blotlike karyosomes visible when mature; cysts measure 5–10 μm and have no visible chromatoidal bars.

Life Cycle

The life cycle of *E. nana* is similar to that described for *E. histolytica*, without an extra-intestinal phase.

Transmission and Pathogenesis

Infection with *E. nana* occurs after ingestion of cysts in contaminated food or water, and results in asymptomatic infection.

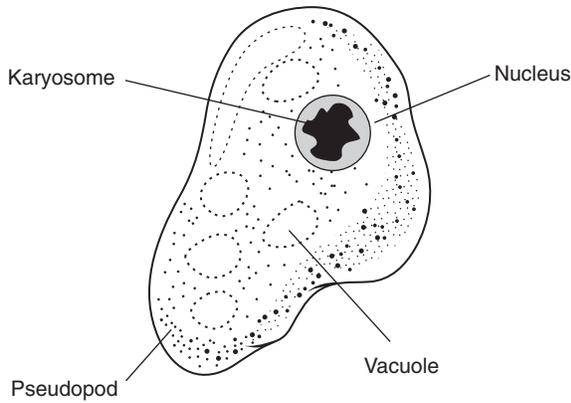


FIG 4-8 *Endolimax nana* trophozoite

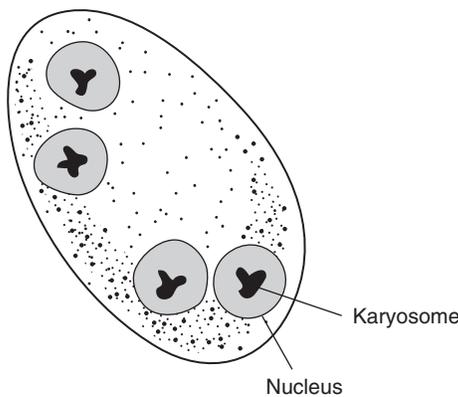


FIG 4-9 *Endolimax nana* cyst

Laboratory Diagnosis

Diagnosis of infection is made by examination of fecal specimens for parasites.

Treatment and Prevention

No treatment is necessary; prevention involves avoidance of contaminated food and water.

Iodamoeba butschlii

Morphology

Trophozoite: The *Iodamoeba butschlii* trophozoite (Figure 4-10) measures 8–20 μm, with coarsely vacuolated cytoplasm containing debris, including bacteria and yeast. A large karyosome usually fills much of the intranuclear space. Chromatin granules radiating from the karyosome may result in the “basket nucleus” appearance of this parasite. No peripheral chromatin is seen. Motility is sluggish and non-progressive.

Cyst: The cyst measures 5–20 μm, with no visible chromatoidal bars (Figure 4-11). It frequently contains

a large glycogen vacuole, which, when fresh, stains reddish brown with iodine. In permanent stains, the glycogen vacuole appears as a clear unstained space. The cyst contains a single nucleus, which is seldom seen in iodine stained preparations. If seen in permanent stained smears, a large karyosome may be present in an eccentric position.

Life Cycle

The life cycle of *I. butschlii* resembles that of other nonpathogenic amebae, although no nuclear multiplication occurs in the cyst form. Therefore the mature cyst contains only a single nucleus.

Transmission and Pathogenesis

The infection is asymptomatic and is transmitted by the fecal-oral route.

Laboratory Diagnosis

Diagnosis is by the examination of fecal specimens for the parasite.

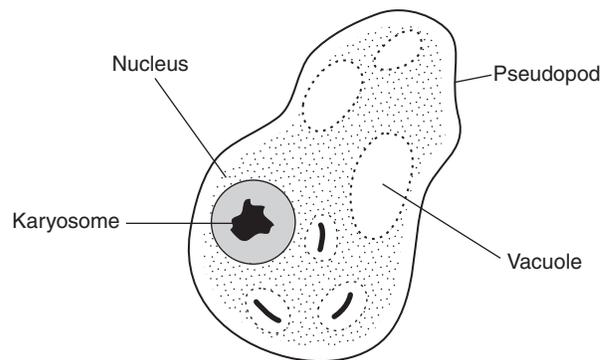


FIG 4-10 *Iodamoeba butschlii* trophozoite

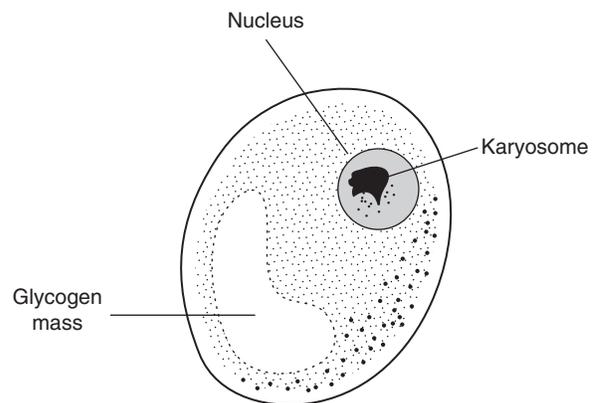


FIG 4-11 *Iodamoeba butschlii* cyst

Treatment and Prevention

No treatment is necessary; infection is prevented by avoiding contaminated food and water.

Blastocystis hominis

Morphology

The **central body** (a clear, transparent area resembling a vacuole) form is usually observed in stool specimens. The spherical to oval vacuolated form measures 6–15 μm , and appears refractile in wet mounts. A clear central area, resembling a large vacuole, is surrounded by 3 to 7 granules around the periphery (Figure 4–12). The iodine stain emphasizes the peripheral granules.

Life Cycle

Although the life cycle of *B. hominis* has not been fully described, stages appear to include the amebic form, the cyst form, and the central body or vacuolated form. The latter stage is the one usually found in human feces, although the amebic form may occasionally be seen in diarrheal stool specimens. The parasite reproduces by binary fission or sporulation; the central body is also involved in sexual and asexual reproduction. *B. hominis* feeds on bacteria and debris in the intestine.

Transmission and Pathogenesis

Although commonly found as a normal resident of the intestinal tract, *Blastocystis hominis* is considered to be a potential pathogen, if present in large numbers. In patients with no other obvious reason for their diarrhea, nausea, abdominal pain or vomiting, moderate numbers of this parasite are believed to be responsible for their symptoms. It has been suggested, however, that these patients may have an additional

undetected pathogen that is responsible for their symptoms. The mode of transmission is not known; however, it is assumed that the infection is acquired by ingesting contaminated food or water.

Laboratory Diagnosis

Infection with *B. hominis* is detected by examination of stool specimens for the presence of characteristic vacuolated forms. A permanent stained smear, such as the trichrome stain, is the preferred method for detection of this parasite, although iodine stained smears are also used to make a diagnosis.

Treatment and Prevention

When *B. hominis* is present in moderate to high numbers in symptomatic patients, the treatment of choice is metronidazole. This agent is also effective against other protozoa. An alternate choice is iodoquinol.

SUMMARY

Although six species of amebae may cause intestinal infection in humans, *E. histolytica* is the only true pathogen in this group. As well as causing amebic dysentery, this parasite may spread beyond the intestine, causing abscesses in the liver and other organs. Infection with nonpathogenic amebae may represent amebiasis spread by the fecal-oral route. Only *E. histolytica* infection requires treatment. Therefore it is imperative to differentiate this parasite from the other commensals in this group. Key characteristics of the trophozoites and cysts of intestinal amebae are summarized in Table 4–1.

Although the pathogenicity of *Blastocystis hominis* has been controversial, it is currently believed that, in the absence of other pathogens, large numbers of this parasite may be responsible for symptoms of gastroenteritis.

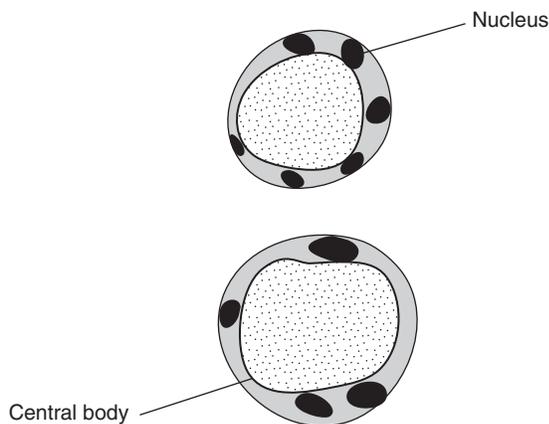


FIG 4–12 *Blastocystis hominis*

Table 4–1 ► Key Characteristics of Intestinal Amebae

AMEBAE	TROPHOZOITE	CYST
<i>Entamoeba histolytica</i>	10–60 μm Finely granular cytoplasm Evenly distributed peripheral nuclear chromatin Small, central, compact karyosome Inclusions: ingestion of RBC is diagnostic Progressive motility	10–20 μm Mature cyst contains 4 nuclei Elongated chromatoidal bars with blunt, smooth ends Small, central, compact karyosome
<i>Entamoeba hartmanni</i>	5–12 μm Finely granular cytoplasm Evenly distributed peripheral nuclear chromatin Central, compact karyosome Inclusions: bacteria, no RBC Non-progressive motility	5–10 μm Mature cyst contains 4 nuclei Elongated chromatoidal bars with blunt, smooth ends Central, compact karyosome
<i>Entamoeba coli</i>	15–50 μm Coarsely granular cytoplasm Unevenly distributed peripheral nuclear chromatin Large, discrete, eccentric karyosome Inclusions: bacteria, yeast, debris Sluggish, non-progressive motility	10–35 μm Mature cyst contains 8 nuclei Splinter shaped chromatoidal bars with sharp, pointed ends Large, eccentric karyosome
<i>Endolimax nana</i>	6–12 μm Granular cytoplasm No peripheral chromatin Karyosomes are large and blotlike Inclusions: bacteria Sluggish, non-progressive motility	5–10 μm Mature cyst contains 4 nuclei No peripheral chromatin Large karyosomes, but smaller than trophozoite No visible chromatoidal bars
<i>Iodamoeba butschlii</i>	8–20 μm Granular, vacuolated cytoplasm No peripheral chromatin Large karyosomes Inclusions: bacteria, yeast Sluggish, non-progressive motility	5–20 μm Mature cyst contains 1 nucleus No peripheral chromatin Large karyosome, with granules on 1 side No visible chromatoidal bars Prominent glycogen vacuole

REVIEW QUESTIONS

- The most significant pathogen among the amebae is:
 - Entamoeba coli*
 - Endolimax nana*
 - Entamoeba histolytica*
 - Entamoeba bartmanni*
- Ingestion of red blood cells is characteristic of:
 - E. histolytica*
 - E. bartmanni*
 - Blastocystis hominis*
 - Iodamoeba butschlii*
- A _____ is a clump of genetic material found in the nucleus of amebae.
 - pseudopod
 - karyosome
 - chromatoid body
 - cyst
- Peripheral nuclear chromatin is *absent* in:
 - E. nana*
 - E. histolytica*
 - E. bartmanni*
 - E. coli*
- A total of 16 nuclei may be found in the ameba:
 - E. histolytica*
 - E. nana*
 - E. coli*
 - E. bartmanni*
- Nonpathogenic strains of _____ are morphologically indistinguishable from *E. histolytica*.
 - Entamoeba dispar*
 - E. coli*
 - E. bartmanni*
 - E. nana*
- Large, blotlike karyosomes are found in:
 - E. dispar*
 - E. coli*
 - E. bartmanni*
 - E. nana*
- A large glycogen vacuole, usually appearing as a clear space in permanent stains, is characteristic of:
 - Endolimax nana*
 - Iodamoeba butschlii*
 - Blastocystis hominis*
 - Entamoeba bartmanni*
- Amebiasis may be transmitted by:
 - food
 - water
 - sexual activity
 - all of the above
- Extra-intestinal infection may occur with infections caused by:
 - E. coli*
 - E. bartmanni*
 - E. nana*
 - E. histolytica*

