CHAPTER 1

Amoeba and Ciliates

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1.1 PREFACE

Amoeba and ciliates are two groups of protozoan parasites that have long been known to infect humans. The amoeba are unicellular organisms which are characterized by the pseudopodia, which are cytoplasmic protrusions that provide motility to the organism. Amoeba are commonly found in the environment and a few are pathogenic to mammals.

The ciliates are protozoa, unicellular organisms that use the cilia on their surface for high motility. Ciliates are commonly found in environmental waters. The only species pathogenic to humans is *Balantidium coli*, which is also found to infect pigs and nonhuman primates.

Amoeba and ciliates can be acquired either by ingestion of contaminated water or food or by contamination of products or surfaces by food handlers.

1.2 AMOEBA

This group of parasites belong to the phylum Sarcomastigophora, subphylum Sarcodina (Bruckner, 1992). The cyst and trophozoite are the two morphological stages of the amoeba. Some amoeba (commensal) can infect humans, but do not cause illness. The free-living amoeba are frequently found in the environment, particularly water sources, but under certain circumstances they can infect humans. Three of public health relevance are *Acantamoeba*, *Naegleria*, and *Balamuthia*. Other amoeba can be identified as infecting humans, but not necessarily causing illness. Some of these commensal amoeba include the genera *Entamoeba*, *Endolimax*, and *Iodamoeba. Blastocystis* has been traditionally considered an amoeba, but has now been reclassified as fungi. The amoeba in humans is most often found to infect the buccal cavity or the gastrointestinal tract. *Entamoeba gingivalis* is commonly associated with gingivitis and is localized in the soft tartar between the teeth and the oral mucosa. It does not have a cyst stage and transmission is considered to be person to person or by contact with buccal secretions.

Nonpathogenic amoeba (commensal) that colonize the intestinal tract include *Entamoeba dispar*, *Entamoeba hartmanni*, *Entamoeba moshkovskii*, *Entamoeba polecki*, *Endolimax nana*, *Entamoeba chattoni*, *Entamoeba invadens*, *Iodamoeba butschlii*, and *Entamoeba coli*.

Amoeba can be identified by observing the morphology of trophozoites or cysts. Trophozoites can be observed only in fresh specimens from an infected individual. The arrangement, size, and pattern of the nuclear chromatin aid in the identification of the various species. The size and position of the karyosome also aid in the

speciation of amoeba. The cytoplasm of the trophozoites may contain red blood cells, bacteria, yeasts, and molds. The number and size of the nuclei in the cyst are taken into consideration when identifying the genera and species of amoeba. Chromatoidal bodies and vacuoles present in the cytoplasm also aid in the identification of the species. All of these characteristics are not easily noted in fresh preparations, requiring permanent stains of fecal smears to be prepared and examined at 1000× magnification. Mixed infections are very common; therefore, observation of several parasitic structures is necessary for a conclusive diagnosis (Leber and Novak, 2005).

The pathogenic amoeba for humans is *Entamoeba histolytica*. It was described by Fedor Losch in 1875 from a Russian patient with dysenteric stools (Lösch, 1875). *E. histolytica* has been recovered worldwide and is more prevalent in the tropics and subtropics than in cooler climates. In areas of temperate and colder climates, it can be found in unsanitary conditions.

The pathogenicity of *Entamoeba* has been controversial. In some instances, *E. histolytica* may cause invasive disease and extraintestinal amebiasis, and in other instances, it may cause mild or asymptomatic infections. The host immune status, strain variability, environmental conditions, and the intestinal flora composition are factors that may influence the clinical presentation of the disease. Axenic cultivation of the amoeba has facilitated the study of isoenzyme profiles including glucophosphate isomerase, phosphoglucomutase, malate dehydrogenase, and hexokinase in various isolates. Sargeaunt concluded that *Entamoeba* could be characterized based on their isoenzyme analysis and characterized in various zymodemes. Of the amoeba that infects humans, *E. dispar* (nonpathogenic) and *E. histolytica* (pathogenic) are not only genotypically different, but phenotypically distinct, although they are morphologically similar.

The life cycle of amoeba starts when the cyst, which is the infectious form, is acquired by ingestion of contaminated materials, such as food and water, or by direct fecal-oral transmission. Once in the intestinal tract, excystation occurs, trophozoites are released and propagate via asexual multiplication. Cyst formation occurs in the colon where conditions are unfavorable for the trophozoite. Cysts are excreted in the feces and can remain viable in the environment for up to several weeks if protected from environmental conditions (Garcia, 1999).

1.2.1 *Entamoeba histolytica*

Entamoeba histolytica has been described worldwide. In areas of endemnicity up to 50% of the population may be infected. It ranks second in worldwide causes of morbidity by parasitic infections (Laughlin and Temesvari, 2005). Humans are the primary reservoirs of this parasite; however, it has been described as infecting nonhuman primates. This transmission can occur via person to person or by ingestion of cysts present in contaminated food or water. The cysts excyst in the intestine and trophozoites are released and start dividing. Some will encyst and be excreted with the feces. In invasive amebiasis, trophozoites may penetrate the bowel and disseminate to the liver, lungs, brain, pericardium, and other tissues. Invasive amebiasis tends to affect men predominantly, but asymptomatic infection is equally distributed among both genders (cuna-Soto *et al*., 2000). Immigrants from South and Central America and Southeast Asia are two groups with a high incidence of amebiasis.

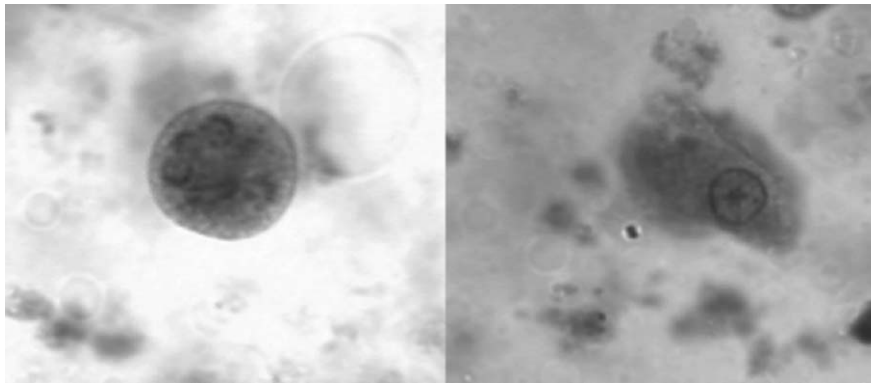

Figure 1.1. *Entamoeba histolytica* (left) cyst and (right) trophozoite.

Travelers are at high risk for acquiring the infection. In areas where *E. histolytica* and *E. dispar* are endemic, *E. histolytica* are more predominant in travelers and *E. dispar* are more predominant in residents. Amebiasis in homosexual males is frequently transmitted by sexual behavior. Asymptomatic presentation is up to 30% (Walderich *et al*., 1997).

1.2.1.1 Morphology

Trophozoites are between 12 and 60 μm in diameter. The nucleus is characterized by evenly arranged chromatin on the nuclear membrane and a karyosome that is small, compact, and centrally located (Fig. 1.1). The cytoplasm is granular and has vacuoles containing bacteria or debris. In cases of dysentery, red blood cells may be present in the cytoplasm. Immature cysts are characterized by one to two nuclei, a glycogen mass, and chromatoidal bars with smooth round edges. Mature cysts have four nuclei, and the glycogen mass and the chromatoidal bars may disappear as the cyst matures. This process occurs while oocysts migrate in the intestine. The cyst measures 10–20 μm. Once the cyst is ingested by the new host, the gastric enzymes and neutral or alkaline pH in the intestine induce the trophozoites to become active, at which point they are liberated (Fig. 1.2).

1.2.1.2 Clinical Significance

The World Health Organization estimates 50 million infections and 100,000 deaths per year (Anonymous, 1997). The clinical presentation of *E. histolytica* can be asymptomatic, symptomatic without tissue invasion, and symptomatic with tissue invasion. Asymptomatic infection may be related to two genetically distinct invasive and noninvasive strains of *E. histolytica* (Zaki and Clark, 2001). Approximately 10% of infected individuals will have clinical symptoms such as dysentery, colitis, or in few instances, amebomas. Amebomas are localized granulomatous tissues with tumor-like lesions resulting from chronic ulceration. They may be mistaken for malignancy. Amoebic dysentery is characterized by diarrhea with cramping, lower abdominal pain, low fever, and the presence of blood and mucus in feces. Ulcers start at the surface of the epithelium that deepens into a classic flask-shaped ulcer.

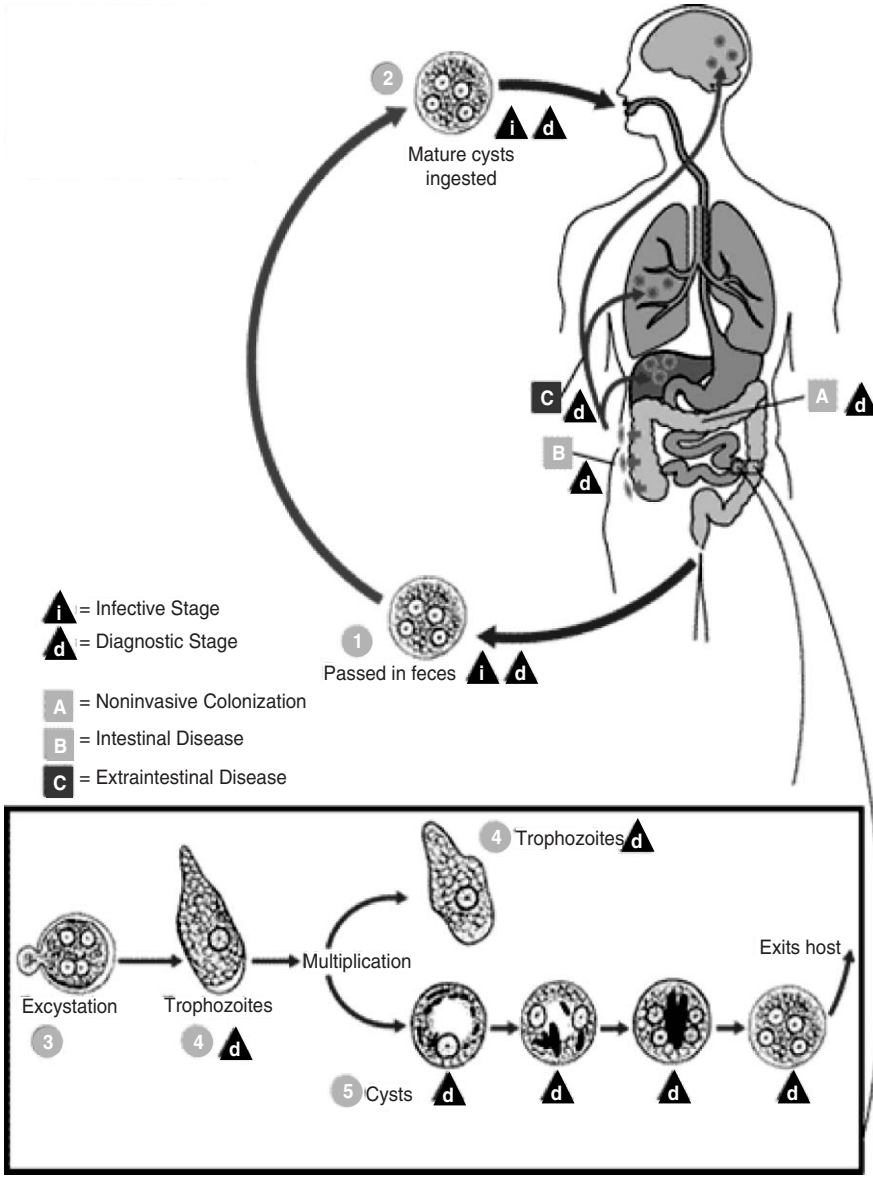

Figure 1.2. *Entamoeba histolytica* life cycle. Graph obtained from http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary

Abdominal perforation and peritonitis are rare, but can be serious complications. Amoebic colitis is characterized by intermittent diarrhea over a long period of time and can be misdiagnosed as ulcerative colitis or irritable bowel syndrome (Leber and Novak, 2005). Incubation period may vary from days to months.

If extraintestinal localization occurs, the liver is the most common site, but infection can also invade the lungs, pericardium, brain, etc. Symptoms may be acute or gradual and may include low-grade fever, pain in the right upper quadrant, and weight loss.

1.2.1.3 Pathogenesis and Immunity

Adhesins, amoebapores, and proteases have been associated with lysis of the colonic mucosa in intestinal amebiasis (Espinosa-Cantellano and Martinez-Palomo, 2000). *Entamoeba* has a cell surface protein that has a sensory activity and contributes to the surface adhesion of the trophozoite. The Gal/GalNAc lectin recognizes galactose and *N*-acetylgalactosamine found on the human colonic mucin glycoproteins. Interaction between this lectin and the host glycoproteins is required for adherence and contact-dependent cytolysis (Petri *et al*., 1989). This lectin is unique in *E. histolytica* and has been used to develop the ELISA diagnostic assay produced by TechLab. The trophozoite moves forming the pseudopod in front, and the membrane moves to the uroid, which is a posterior foot. The amoeba collects surface antigens, including host antibodies, on the uroid. Membrane shedding is active at the uroid region, eliminating the accumulated ligands including antigens, Gal/GalNAc lectins, and the 96-kDa surface protein with the host antibodies. This process may contribute to the evasion of the host immune defenses. Amoeba with a defective cytoskeleton cannot form a cap or form uroids, and cannot cause cell cytolysis, suggesting that the cytoskeleton may play a role in contact-dependent cytolysis (Arhets *et al*., 1998).

The cysteine proteinases are a major virulence factor. These proteinases can degrade elements of the extracellular matrix including fibronectin, laminin, and type I collagen (Keene *et al*., 1986). These proteinases also interfere with the complement pathways and the humoral response of the human immune system. Gal/GalNAc lectin inhibits complement-mediated lysis because it mimics the CD59, a membrane inhibitor of C5b-9 in human blood cells. The proteinases can degrade and inactivate C3 and C5 to circumvent the host immune response, as well as degrade secretory IgG and IgA, limiting the host humoral immune response (Kelsall and Ravdin, 1993). The presence of IgA antilectin provides a marker of acquired immunity.

E. histolytica also secretes a pore-forming protein, the amoebapore containing three isoforms: A, B, and C. It works by inserting ion channels into artificial membranes and may be cytolytic to eukaryotic cells (Leippe *et al*., 1994; Rosenberg *et al*., 1989).

The mechanisms of host defense include production of mucin. The Gal/GalNac lectin binds to it. Whether it serves as defense or as an inducer for colonization needs to be determined (Petri *et al*., 1989).

The inflammatory response provides another mechanism of defense. *In vitro* and *in vivo* studies demonstrated that the presence of trophozoites causes the expression of a variety of cytokines, including IL-1b and IL-8. This production occurred in regions other than those in direct contact with the parasite (Zhang *et al*., 2000).

A subunit of the Gal/GalNAc lectin of 170 kDa induces production of IL-12 in human macrophages. The IL-12 promotes Th1 cytokine differentiation and, in turn, macrophage protection (Campbell *et al*., 2000).

Diagnosis can be made by examination of fecal samples, material collected using a sigmoidoscope, tissue biopsy, and abscess aspirates. Serological testing can be used. Serum antibodies have been identified in 85% of patients with proven amebiasis (by histology) and in 99% of patients with extraintestinal amebiasis. Persons with *E. dispar* do not develop detectable levels of antibodies (Leber and Novak, 2005). Diagnosis is facilitated by the examination of permanently stained slides. Diagnostic assays specific for *E. histolytica* in clinical specimens are available on the market (TechLab, Blacksburg, VA) (Garcia *et al*., 2000; Ong *et al*., 1996; Pillai *et al*., 1999). Zymodeme analysis has been used to differentiate between *E. histolytica* and *E. dispar*; which, although specific, is also expensive and time-consuming.

Molecular assays such as polymerase chain reaction (PCR) have been developed, but do not seem to be very sensitive when compared to conventional assays (Evangelopoulos *et al*., 2000; Rivera *et al*., 1996; Sanuki *et al*., 1997; Zindrou *et al*., 2001). Roy and collaborators compared a real-time PCR against the antigen detection tests and SS- rRNA and traditional PCR (72% sensitive and 99% specific). The real-time PCR was more sensitive (79% sensitive and 96% specific) than all the other assays and the specificity was higher by PCR. Using the TechLab antigen, detection kit detected only 49% of positive specimens (Roy *et al*., 2005).

1.2.1.4 Therapy

If treating asymptomatic infection with cyst excretion, a luminal amoebicide such as iodoquinol or diloxanide furoate is recommended. If tissue invasion has occurred, tissue amoebicides such as metronidazole, chloroquine, or dehydroemetine are recommended. Follow-up stool examination is important, since these treatments may lead to drug resistance. The multidrug resistance gene *EhPgp1* is constitutively expressed in drug-resistant trophozoites (Ramirez *et al*., 2005).

1.3 *DIENTAMOEBA FRAGILIS*

Dientamoeba, originally considered an amoeba, is now considered an amoeba-flagellate and is closely related to *Histomonas* and *Trichomonas* spp.

1.3.1 Morphology and Transmission

The trophozoite measures 5–15 μm and pseudopodia are angular. No flagella is present. The cytoplasm is highly granular and it is characterized as having one to two nuclei without peripheral chromatin and karyosome clusters of four to eight granules. Cysts have not been identified in *Dientamoeba fragilis*. This amoeba-flagellate does not have a cyst form and its transmission is less understood. However, transmission is suspected to be associated with helminth eggs such as *Acaris* and *Enterobius*. Higher incidences have been reported in mental institutions, missionaries, and Indians in Arizona. It has been reported in pediatric populations (Anonymous, 1993). Symptoms include fatigue, intermittent diarrhea, abdominal pain, anorexia, and nausea. It has been reported to cause noninvasive diarrheal illness. *Dientamoeba* colonizes the cecum and the proximal part of the colon. Reports of *Dientamoeba* are limited

and this may be related to the difficulty in identifying the organisms. Asymptomatic cases of *D. fragilis* have been reported. This may be related to the description of two genetic variants using PCR-RFLP of the ribosomal genes (Johnson and Clark, 2000).

1.3.2 Therapy

Tetracycline or iodoquinol are recommended as the drug of choice for individuals with symptomatic infection. If co-infections include helminths such as *Enterobius*, mebendazole is usually included in the treatment (Butler, 1996).

1.4 NONPATHOGENIC AMOEBA

1.4.1 *Entamoeba hartmanni*

E. hartmanni is morphologically similar to *E. histolytica/E. dispar*. The trophozoite measures 5–12 μm and has one nucleus with a peripheral chromatin. The karyosome is small, compact, and centrally located. The cyst measures 5–10 μm. The mature cyst contains four nuclei. Chromatoidal bodies are like those of *E. histolytica*.

1.4.2 *Entamoeba coli*

It is commonly found in individuals in developing countries. It is characterized by having a cyst of 10–35 μm that may contain up to eight nuclei. Chromatoidal bars are splinter shaped and have rough pointed ends. The nuclei have distinctive characteristics, including the coarsely granular peripheral chromatin. The large karyosome is usually eccentric. The trophozoite measures between 15 and 50 μm and bacteria are usually present in the cytoplasm.

1.4.3 *Endolimax nana*

The trophozoite measures between 6 and12 μm and has a granulated and vacuolar cytoplasm. The cyst measures between 5 and 10 μm. It is usually oval and when mature may have up to four nuclei. The nuclei have nonvisible peripheral chromatin and the karyosome is larger than the *Entamoeba.* Morphologically, it is very different from the *Entamoeba* species.

1.4.4 *Iodamoeba butschlii*

The trophozoite measures between 8 and 20 μm. The cytoplasm is granular and vacuolated. The cyst may be oval or round and measures between 5 and 20 μm. The mature cyst, contrary to the other amoeba, contains only one nucleus characterized by the absence of peripheral chromatin and a larger karyosome. It usually contains a large glycogen vacuole that stains brown when the sample is prepared using iodine. *Iodamoeba* can be easily differentiated from the other amoeba.

E. coli, E. nana, and *I. butschlii* can be easily differentiated from *E. histolytica* primarily by their size, followed by the nuclei characteristics and cytoplasmic inclusions.

1.5 FREE-LIVING AMOEBAE

Naegleria, *Acanthamoeba*, and *Balamuthia* have been identified in the central nervous system of humans and other animals. *Acanthamoeba* can also cause keratitis, and both *Acanthamoeba* and *Balamuthia madrillaris* may cause cutaneous infection in humans. *Naegleria fowleri* and *Acanthamoeba* spp. are commonly found in soil, water, sewage, and sludge. These amoebae feed on bacteria and multiply in the environment. They may harbor pathogenic bacteria to humans such as *Legionella*, *Mycobacterium avium*, *Listeria*, etc. Whether *Acanthamoeba* serves as a reservoir for human pathogens is unknown. Meningoencephalitis caused by *Naegleria* has been coined primary amebic meningoencephalitis. It is an acute and fulminant disease that can occur in previously healthy children and young adults who have been in contact with freshwater about 7–10 days prior to development of clinical signs. It is characterized by severe headache, spiking fever, stiff neck, photophobia, and coma, leading to death within 3–10 days after onset of symptoms. The amoeba find their way through the nostrils, to the olfactory lobes and cerebral cortex.

Acanthamoeba and *Balamuthia* encephalitis are found primarily in immunosuppressed individuals who have had exposure to recreational freshwater. Chronic granulomatous amoebic encephalitis (GAE) has an insidious onset and is usually chronic. The invasion and penetration to the central nervous system may be through the respiratory tract or the skin. These amoebae have been predominantly associated with waterborne transmission in recreational waters. Whether these amoebae are associated with foodborne transmission has not been determined.

N. fowleri is susceptible to amphotericin B alone or in combination with miconazole. Few patients infected with *Acanthamoeba* have survived when treated, but in most instances, patients with encephalitis have died. A successful recovery of a patient with GAE included surgery and treatment with sulfadiazine and fluconazole (Seijo *et al*., 2000). Skin infections have a good prognosis and usually require topical treatment with 2% ketoconazole cream.

1.6 CILIATES

Ciliates are highly motile protozoa. They are characterized by cilia present on the surface. Free-living ciliates can be found in environmental waters. The only species pathogenic to humans is *B. coli*. It was initially identified in dysenteric stools of two patients and was later described by Leukhart in 1861 and Stein in 1862 (Diana, 2003). *Balantidium* can exist in reservoirs such as pigs and nonhuman primates. *B. coli* can be found in as many as 45% of pigs from intensive farming to 25% in wild boars (Solaymani-Mohammadi *et al*., 2004; Weng *et al*., 2005). In Denmark, 57% of suckling pigs had *Balantidium* (Hindsbo *et al*., 2000); however, balantidiasis has not been reported in humans. In some regions of Venezuela, balantidiasis was observed to be 12% in humans and 33.3% in pigs. Nonhuman primates have also been reported carrying the infection in the tropics. Monkeys, chimpanzees, gibbons, macaques, and gorillas can harbor *Balantidium* (Nakauchi, 1999).

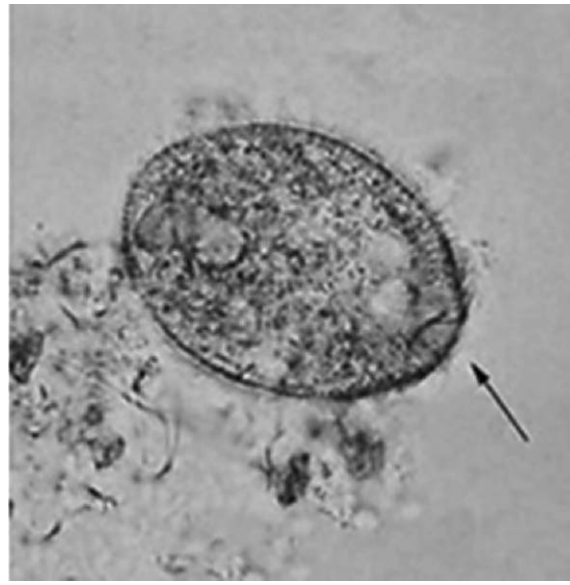

Figure 1.3. *Balantidium coli* trophozoite. Arrow points at the trophozoite cytostome. Picture obtained from http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary

Human infections can occur in warmer climates. Sporadic cases have been reported in cooler areas and in institutionalized groups with poor hygienic conditions. In the United States it is rarely found in clinical specimens. Deficient environmental sanitation favor dissemination of the infection (Devera *et al*., 1999).

1.6.1 Life Cycle and Morphology

The trophozoite and the cyst are the only two stages of *B. coli*. The trophozoite is large and oval. It measures 50–100 μm long to 40–70 μm wide. The cyst measures 50–70 μm. The movement is rotary. The body is covered with longitudinal rows of cilia and they are longer near the cytostome. The trophozoite is pear shaped with an anterior end pointed and the posterior end broadly rounded. The cytoplasm contains vacuoles with ingested bacteria and cell debri. The trophozoite and cyst contain two nuclei: one large bean-shaped nucleus and a round micronucleus (Fig. 1.3). The cyst form is the infective stage. It has a thick cyst wall. Trophozoites secrete hyaluronidase, which aids in the invasion of the tissues. Cysts are formed as the trophozoite moves down the large intestine (Fig. 1.4).

1.6.2 Clinical Significance

Frequently, *Balantidium* infections can be asymptomatic; however, severe dysentery similar to those with amoebiasis may be present. Symptoms include diarrhea or dysentery, tenesmus, nausea, vomiting, anorexia, and headache. Insomnia, muscular weakness, and weight loss have also been reported. Diarrhea may persist for weeks or months prior to development of dysentery. Fluid loss is similar to that

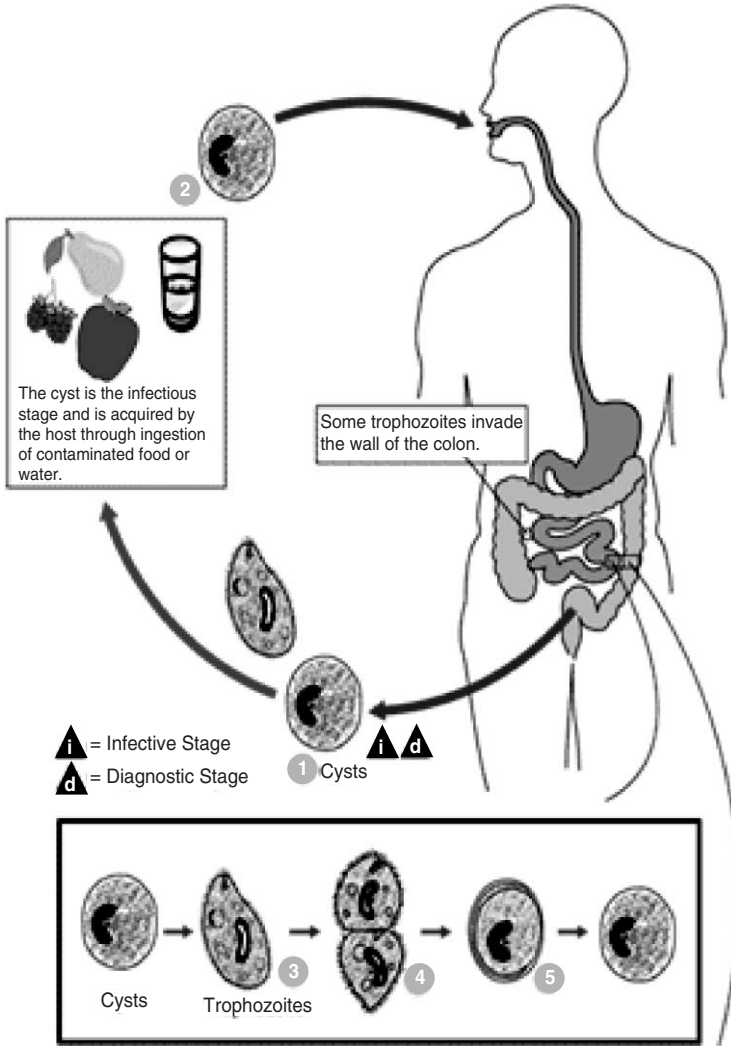

Figure 1.4. *Balantidium coli* life cycle diagram. Picture obtained from http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary

observed in cholera or cryptosporidiosis. Symptomatic infections can occur, resulting in bouts of dysentery similar to amebiasis. Colitis caused by *Balantidium* is often indistinguishable from *E. histolytica* (Castro *et al*., 1983). Diarrhea, nausea, vomiting, headache, and anorexia are characteristic of balantidiasis.

The organism can invade the submucosa of the large bowel, causing ulcerative abscess and hemorrhagic lesions to occur. The shallow ulcers are prone to secondary infections by bacteria and can be problematic for the patient (Knight, 1978b). In few cases, extraintestinal disease such as peritonitis, urinary tract infection, and

inflammatory vaginitis has been reported. *Balantidium* has been described in the urinary bladder of an infected individual (Knight, 1978a; Ladas *et al*., 1989; Maleky, 1998). Pulmonary lesions can occur in immunocompromised patients without obvious contact with pigs, nor history of diarrhea prior to pulmonary infection (Anargyrou *et al*., 2003). *Balantidium* pneumonia has been described in a 71-year-old woman suffering from anal cancer (Vasilakopoulou *et al*., 2003). Chronic colitis and inflammatory polyposis of the rectum and sigmoid colon and an intrapulmonary mass have been described in a case with balantidiasis (Ladas *et al*., 1989).

After ingestion, the trophozoite excretes hyaluronidase, which aids in the invasion of the tissue. On contact with mucosa, mucosal invasion is accomplished by cellular infiltration in the area of the developing ulcer. The organism can invade the submucosa of the large bowel. Ulcerative abscesses and hemorrhagic lesions can occur. Some of the abscess formations may extend to the muscular layer. The shallow ulcers and submucosal lesions are prone to secondary bacterial infection. Ulcers may vary in shape and the ulcer bed may be full of mucus and necrotic debris.

1.6.3 Diagnosis and Treatment

Wet preparation examinations of fresh and concentrated fecal material can determine the organism, as the shape and motility are characteristic of this ciliate. Tetracycline is the drug of choice, although it is considered an investigational drug for this infection. Iodoquinol or metronidazole may be used as alternative drugs.

1.6.4 Epidemiology and Prevention

Several studies have demonstrated the presence of *B. coli* in developing countries. Balantidiasis has been reported in 8% of children of the Bolivian Altiplano (Basset *et al*., 1986).

Domestic hogs probably serve as the most important reservoir host for balantidiasis. In areas where pigs are the main domestic animal, the incidence of infection is high. Risk factors to acquire this infection include working at pig farms or slaughterhouses. Infection can turn into an epidemic if conditions favor propagation in the community. This has been observed in mental hospitals in the United States, where poor sanitary conditions are common. Preventive measures include increased attention to personal hygiene and sanitation measures, since the mechanisms of transmission are via contaminated water or foods with *Balantidium* cysts.

REFERENCES

Anargyrou, K., Petrikkos, G. L., Suller, M. T., Skiada, A., Siakantaris, M. P., Osuntoyinbo, R. T., Pangalis, G., and Vaiopoulos, G., 2003, Pulmonary *Balantidium coli* infection in a leukemic patient, *Am. J. Hematol.* **73**:180–183.

Anonymous, 1993, Intestinal protozoa: Flagellates and ciliates. In Garcia, L. S. and Bruckner, D. A. (eds), *Diagnositc Medical Parasitology*, Vol. 3, American Society for Microbiology, Washington, DC, pp. 31–44.

Anonymous, 1997, WHO/PAHO/UNESCO report. A consultation with experts on amoebiasis. Mexico City, Mexico 28–29 January, 1997, *Epidemiol. Bull.* **18**: 13–14.

Arhets, P., Olivo, J. C., Gounon, P., Sansonetti, P., and Guillen, N., 1998, Virulence and functions of myosin II are inhibited by overexpression of light meromyosin in *Entamoeba histolytica*, *Mol. Biol. Cell* **9**:1537–1547.

Basset, D., Gaumerais, H., and Basset-Pougnet, A., 1986, Intestinal parasitoses in children of an Indian community of Bolivian altiplano, *Bull. Soc. Pathol. Exot. Filiales.* **79**:237–246.

Bruckner, D. A., 1992, Amebiasis, *Clin. Microbiol. Rev.* **5**:356–369.

Butler, W. P., 1996, *Dientamoeba fragilis*. An unusual intestinal pathogen, *Dig. Dis. Sci.* **41**:1811–1813.

Campbell, D., Mann, B. J., and Chadee, K., 2000, A subunit vaccine candidate region of the *Entamoeba histolytica* galactose-adherence lectin promotes interleukin-12 gene transcription and protein production in human macrophages, *Eur. J. Immunol.* **30**:423–430.

Castro, J., Vazquez-Iglesias, J. L., and rnal-Monreal, F., 1983, Dysentery caused by *Balantidium coli*—report of two cases, *Endoscopy* **15**:272–274.

cuna-Soto, R., Maguire, J. H., and Wirth, D. F., 2000, Gender distribution in asymptomatic and invasive amebiasis, *Am. J. Gastroenterol.* **95**:1277–1283.

Devera, R., Requena, I., Velasquez, V., Castillo, H., Guevara, R., De, S. M., Marin, C., and Silva, M., 1999, Balantidiasis in a rural community from Bolivar State, Venezuela, *Bol. Chil. Parasitol.* **54**:7–12.

Diana, E., 2003, Intestinal parasitoses: A history of scientific progress and endemic disease in society. In Dionisio, D. (ed.), *Atlas of Intestinal Infections in AIDS*, Vol. 1, Springer-Verlag Italia, Milan, pp. 7–34.

Espinosa-Cantellano, M., and Martinez-Palomo, A., 2000, Pathogenesis of intestinal amebiasis: From molecules to disease, *Clin. Microbiol. Rev.* **13**:318–331.

Evangelopoulos, A., Spanakos, G., Patsoula, E., Vakalis, N., and Legakis, N., 2000, A nested, multiplex, PCR assay for the simultaneous detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in faeces, *Ann. Trop. Med. Parasitol.* **94**:233–240.

Garcia, L. S., 1999, Flagellates and ciliates, *Clin. Lab Med.* **19**:621–638, vii.

Garcia, L. S., Shimizu, R. Y., and Bernard, C. N., 2000, Detection of *Giardia lamblia*, *Entamoeba histolytica*/*Entamoeba dispar*, and *Cryptosporidium parvum* antigens in human fecal specimens using the triage parasite panel enzyme immunoassay, *J. Clin. Microbiol.* **38**:3337–3340.

Hindsbo, O., Nielsen, C. V., Andreassen, J., Willingham, A. L., Bendixen, M., Nielsen, M. A., and Nielsen, N. O., 2000, Age-dependent occurrence of the intestinal ciliate *Balantidium coli* in pigs at a Danish research farm, *Acta Vet. Scand.* **41**:79–83.

Johnson, J. A., and Clark, C. G., 2000, Cryptic genetic diversity in *Dientamoeba fragilis*, *J. Clin. Microbiol.* **38**:4653–4654.

Keene, W. E., Petitt, M. G., Allen, S., and McKerrow, J. H., 1986, The major neutral proteinase of *Entamoeba histolytica*, *J. Exp. Med.* **163**:536–549.

Kelsall, B. L., and Ravdin, J. I., 1993, Degradation of human IgA by *Entamoeba histolytica*, *J. Infect. Dis.* **168**:1319–1322.

Knight, R., 1978a, Giardiasis, isosporiasis, and balantidiasis, *Clin. Gastroenterol.* **7**:31–47.

Knight, R., 1978b, Giardiasis, isosporiasis, and balantidiasis, *Clin. Gastroenterol.* **7**:31–47.

Ladas, S. D., Savva, S., Frydas, A., Kaloviduris, A., Hatzioannou, J., and Raptis, S., 1989, Invasive balantidiasis presented as chronic colitis and lung involvement, *Dig. Dis. Sci.* **34**:1621–1623.

Laughlin, R. C., and Temesvari, L. A., 2005, Cellular and molecular mechanisms that underlie *Entamoeba histolytica* pathogenesis: Prospects for intervention, *Expert. Rev. Mol. Med.* **7**:1–19.

Leber, A. L., and Novak, S. M., 2005, Intestinal and urogenital parasites. In Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A., and Yolken, R. H. (eds), *Manual of Clinical Microbiology*, Vol. 133, ASM Press, Washington, pp. 1990–2007.

Leippe, M., Andra, J., Nickel, R., Tannich, E., and Muller-Eberhard, H. J., 1994, Amoebapores, a family of membranolytic peptides from cytoplasmic granules of *Entamoeba histolytica*: Isolation, primary structure, and pore formation in bacterial cytoplasmic membranes, *Mol. Microbiol.* **14**:895–904.

Lösch, F., 1875, Massenhafte entwicklung von amobën im dickdarm. *Archiv für pathologische anatomie und physiologie und für klinsche medicin, von Rudolf Virchow* **65**:196–211.

Maleky, F., 1998, Case report of *Balantidium coli* in human from south of Tehran, Iran, *Indian J. Med. Sci.* **52**:201–202.

Nakauchi, K., 1999, The prevalence of *Balantidium coli* infection in fifty-six mammalian species, *J. Vet. Med. Sci.* **61**:63–65.

Ong, S. J., Cheng, M. Y., Liu, K. H., and Horng, C. B., 1996, Use of the ProSpecT microplate enzyme immunoassay for the detection of pathogenic and non-pathogenic *Entamoeba histolytica* in faecal specimens, *Trans. R. Soc. Trop. Med. Hyg.* **90**:248–249.

Petri, W. A., Jr., Chapman, M. D., Snodgrass, T., Mann, B. J., Broman, J., and Ravdin, J. I., 1989b, Subunit structure of the galactose and *N*-acetyl-D-galactosamine-inhibitable adherence lectin of *Entamoeba histolytica*, *J. Biol. Chem.* **264**:3007–3012.

Pillai, D. R., Keystone, J. S., Sheppard, D. C., MacLean, J. D., MacPherson, D. W., and Kain, K. C., 1999, *Entamoeba histolytica* and *Entamoeba dispar*: Epidemiology and comparison of diagnostic methods in a setting of nonendemicity, *Clin. Infect. Dis.* **29**:1315–1318.

Ramirez, M. E., Perez, D. G., Nader, E., and Gomez, C., 2005, *Entamoeba histolytica*: Functional characterization of the −234 to −196 bp promoter region of the multidrug resistance *EhPgp1* gene, *Exp. Parasitol.* **110**:238–243.

Rivera, W. L., Tachibana, H., Silva-Tahat, M. R., Uemura, H., and Kanbara, H., 1996, Differentiation of *Entamoeba histolytica* and *E. dispar* DNA from cysts present in stool specimens by polymerase chain reaction: Its field application in the Philippines, *Parasitol. Res.* **82**:585–589.

Rosenberg, I., Bach, D., Loew, L. M., and Gitler, C., 1989, Isolation, characterization and partial purification of a transferable membrane channel (amoebapore) produced by *Entamoeba histolytica*, *Mol. Biochem. Parasitol.* **33**:237–247.

Roy, S., Kabir, M., Mondal, D., Ali, I. K., Petri, W. A., Jr., and Haque, R., 2005, Real-time-PCR assay for diagnosis of *Entamoeba histolytica* infection, *J. Clin. Microbiol.* **43**:2168–2172.

Sanuki, J., Asai, T., Okuzawa, E., Kobayashi, S., and Takeuchi, T., 1997, Identification of *Entamoeba histolytica* and *E. dispar* cysts in stool by polymerase chain reaction, *Parasitol. Res.* **83**:96–98.

Seijo, M. M., Gonzalez-Mediero, G., Santiago, P., Rodriguez De, L. A., Diz, J., Conde, C., and Visvesvara, G. S., 2000, Granulomatous amebic encephalitis in a patient with AIDS: Isolation of *Acanthamoeba* sp. Group II from brain tissue and successful treatment with sulfadiazine and fluconazole, *J. Clin. Microbiol.* **38**:3892–3895.

Solaymani-Mohammadi, S., Rezaian, M., Hooshyar, H., Mowlavi, G. R., Babaei, Z., and Anwar, M. A., 2004, Intestinal protozoa in wild boars (Sus scrofa) in western Iran, *J. Wildl. Dis.* **40**:801–803.

Vasilakopoulou, A., Dimarongona, K., Samakovli, A., Papadimitris, K., and Avlami, A., 2003, *Balantidium coli* pneumonia in an immunocompromised patient, *Scand. J. Infect. Dis.* **35**:144–146.

Walderich, B., Weber, A., and Knobloch, J., 1997, Differentiation of *Entamoeba histolytica* and *Entamoeba dispar* from German travelers and residents of endemic areas, *Am. J. Trop. Med. Hyg.* **57**:70–74.

Weng, Y. B., Hu, Y. J., Li, Y., Li, B. S., Lin, R. Q., Xie, D. H., Gasser, R. B., and Zhu, X. Q., 2005, Survey of intestinal parasites in pigs from intensive farms in Guangdong Province, People's Republic of China, *Vet. Parasitol.* **127**:333–336.

Zaki, M., and Clark, C. G., 2001, Isolation and characterization of polymorphic DNA from *Entamoeba histolytica*, *J. Clin. Microbiol.* **39**:897–905.

Zhang, Z., Wang, L., Seydel, K. B., Li, E., Ankri, S., Mirelman, D., and Stanley, S. L., Jr., 2000, *Entamoeba histolytica* cysteine proteinases with interleukin-1 beta converting enzyme (ICE) activity cause intestinal inflammation and tissue damage in amoebiasis, *Mol. Microbiol.* **37**:542–548.

Zindrou, S., Orozco, E., Linder, E., Tellez, A., and Bjorkman, A., 2001, Specific detection of *Entamoeba histolytica* DNA by hemolysin gene targeted PCR, *Acta Trop.* **78**:117–125.