Waterborne diseases associated with polluted recreational and potable waters have been documented for more than a century. Key microbial protozoan parasites, such as Cryptosporidium and Giardia, are causative agents for gastrointestinal disease worldwide. Although not a first-line diagnostic approach for these diseases, medical imaging, such as radiography, computed tomography, magnetic resonance imaging, ultrasonography, and nuclear medicine technologies, can be used to evaluate patients with long-term effects. This article describes protozoan pathogens that affect human health, treatment of common waterborne pathogen-related diseases, and associated medical imaging.

After completing this article, the reader should be able to:

- List the most prevalent protozoan waterborne pathogens.
- Describe the basic life cycle of protozoan waterborne pathogens.
- Explain diseases associated with each protozoan pathogen.
- Compare methods used to identify waterborne pathogen-related disease.
- Discuss challenges and ethical issues regarding waterborne disease.
- Describe the role of medical imaging in waterborne pathogen-related disease.

Waterborne disease is a global problem. According to the World Health Organization (WHO) and a 2014 study, at least 1.8 billion people around the world do not have access to safe drinking water.\(^1,2\) It is estimated that more than half of those people will die as a result, and more than 700,000 children aged 5 and younger will die each year from diarrhea-related diseases caused by ingestion of unsafe water.\(^3,4\) According to a 2015 WHO study, water scarcity, combined with population growth and demographic changes, will result in half of the world’s population residing in water-stressed areas by 2025.\(^5\)

Geographic areas with deteriorating water system infrastructure and inadequate disinfection are likely to encounter increasing incidences of waterborne disease. The extent of the problem is difficult to determine and likely is underestimated because no worldwide monitoring system exists. In the United States, waterborne pathogens are not among the top 10 causes of death.\(^6\)

Pathogens can be one of thousands of types of particles or microorganisms, such as protozoa, bacteria, fungi, viruses, and prions. Pathogens can cause disease in animals, plants, and microorganisms, as well as humans.\(^7\) Their pathways of transmission vary and include zoonotic (i.e., animal- or insect-to-person), foodborne, soilborne, or waterborne transmission. Routes of transmission are not always clear, and some pathogens have several modes of transmission, although usually one way is more common for each type.

Waterborne pathogens are transmitted to humans via contaminated water. Anyone can acquire a waterborne illness, but people with compromised immune systems are at higher risk of contracting a more severe form of any waterborne disease caused by protozoa.\(^7\) Protozoa contribute to significant disease worldwide through waterborne transmission and other routes such as contaminated soil and mosquito bites.

The most prominent subgroup of waterborne diseases is diarrheal
disease, including cryptosporidiosis, giardiasis, cholera, shigellosis, typhoid, hepatitis A, hepatitis E, and poliomyelitis. Diarrhea is a symptom of infections caused by bacterial, viral, and protozoal organisms, and such diseases are responsible for 1.5 million deaths every year, with more than 80% of those deaths attributed to unsafe water and lack of proper sanitation and hygiene. In the past 40 years, waterborne pathogens increasingly have been implicated in the compromised health of millions of people, mostly in developing nations where cryptosporidiosis from Cryptosporidium species causes approximately 10% to 15% of acute diarrhea. Children in these areas have the highest rates of infection, and studies in Brazil have documented an infection rate of approximately 90% in children younger than age 5. In addition, 12% to 48% of people with AIDS have diarrhea as a result of this pathogen.

Cryptosporidium and Giardia are ubiquitous, with numerous outbreaks reported globally. They cause most reported gastrointestinal disease. These parasites can be transmitted to humans in various ways, but transmission via contaminated drinking water and recreational water are the most common. These protozoans are especially problematic in geographic areas with limited resources, and recent global studies have shown these 2 genera to be the most important causes of life-threatening diarrhea in infants and toddlers. Cryptosporidium and Giardia contribute significantly to diarrheal disease morbidity encountered in developing nations and are major concerns in industrialized nations despite improved sanitation; the pathogens are responsible for small-scale and large-scale disease outbreaks. For example, in 1993, more than 400 000 people living in the Milwaukee area were infected with Cryptosporidium when one of the city’s water treatment systems malfunctioned.

Other protozoan pathogens, such as Cyclospora cayetanensis, Entamoeba histolytica, and Naegleria fowleri, are often overshadowed by the number of illnesses caused by Giardia and Cryptosporidium, but they still have a profound affect in various regions. Cyclospora cayetanensis is an enteric pathogen that can cause diarrhea, Entamoeba histolytica is an ameba that lives in the lower intestine but can migrate, causing extraintestinal disease, and Naegleria fowleri, also an ameba, can affect the central nervous system.

Types of Pathogenic Protozoa
In nature, protozoa play a key role in maintaining the balance of bacterial, algal, and other microbial life. These unicellular microorganisms are nonphototrophic, meaning they do not acquire their energy from sunlight. They are eukaryotic, having a membrane-bound nucleus and organelles. Cell walls sometimes are present. Most protozoa range in size from 1/5000 to 1/50 of an inch (5-500 µm) in diameter. Most parasitic protozoa in humans are smaller than 50 µm; the smallest are 1 µm to 10 µm long, although some, such as Balantidium coli, can measure 150 µm. Protozoa also are an important food source for larger creatures and the basis of many food chains. They can be found in almost every environment, and several protozoan species are included in the microbial flora in the gut of insects and mammals. The protozoa help break down complex food particles into simpler molecules for digestion. More than 50 000 species have been described, and of the more than 8000 protozoan species cataloged thus far, most do no harm to humans.

Pathogenic protozoa include:
- Cryptosporidium parvum.
- Cyclospora cayetanensis.
- Blastocystis hominis.
- Dientamoeba fragilis.
- Entamoeba histolytica.
- Giardia duodenalis.
- Naegleria fowleri.

Often, as in the case of species C parvum and G duodenalis, the parasites commonly are referred to by their genera, such as Cryptosporidium and Giardia, and many sources refrain from naming species or genotypes. However, in some cases, such as Cryptosporidium, several species and genotypes exist that are infectious to humans, and their ability to infect relies on various factors, including a person’s genetics and current state of health. Furthermore, it is likely that additional species and genotypes capable of infecting humans have yet to be discovered.

Protozoan classification and interrelationships of major groups of protozoa are not always clear and often
are debated. The protozoan pathogens discussed here can be classified into 4 main subgroups, generally based on their locomotion using specialized subcellular and cytoskeletal features: flagellates, apicomplexans, sarcodines, and ciliates (see Figure 1).

Flagellates are protozoa that possess, at some time in their life cycle, one to many flagella for locomotion and sensation. Flagellates are recognized easily under a microscope because of their size, which ranges from 10 µm to more than 100 µm.21,22 A flagellum is a hair-like structure capable of whip-like lashing movements. Protozoal flagellum is structurally different from bacterial flagellum and waves, whereas bacterial flagellum spins. G duodenalis is a common pathogenic flagellate as is Trypanosoma brucei, which is transmitted by the tsetse fly and causes African Trypanosomiasis, or sleeping sickness. Another common protozoan is Trichomonas vaginalis, a sexually transmitted flagellate that can cause urogenital symptoms in infected women.26

Sporozoa are a large and diverse class with thousands of species.21,22 Unlike the flagellates, sporozoans have no flagellated extensions for locomotion and usually have only gliding motility through use of tiny undulating ridges in the cell membrane. All sporozoans are in the phylum Apicomplexa because of a structure known as an apical complex.23 The apical complex consists of cytoskeletal structures, such as a small open cone, apical rings, and secretory vesicles (eg, rhoptries and micronemes), which allow sporozoans to invade a host’s cells.27 In contrast to the flagellates, the sporozoa reproduction cycle has both asexual and sexual phases.28

Sarcodines are the largest group of protozoans, with 11 500 living species and 33 000 fossil species.29 This subgroup of protozoa includes the heliozoa, radiozoa, foraminifera, and ameba. Sarcodines are categorized according to their pseudopods, or “false feet,” which are used for locomotion and feeding. They include naked forms and various forms with protective shells, also called tests.30 Several sarcodines’ tests are covered with small and large pores through which water can flow and pseudopods can be extended. Sarcodines can replicate asexually by means of cell division or sexually through the production and fusion of gametes and the formation of zygotes. A few sarcodines are pathogenic, such as the ameba Entamoeba histolytica, which was responsible for the Chicago World’s Fair epidemic in 1933.31

Amebae are the most common sarcodines and are found in all environments. Various amebae have a “naked” trophozoite stage in which the cell has no structural components on its membrane that help it maintain a certain shape.32 Many amebae are active predators as opposed to the heliozoa, radiozoa, and foraminifera that rely mainly on suitable food swimming or drifting past. Amebae move and capture food by forming lobe-like pseudopods.33 In other sarcodines, such as radiozoa and foraminifera, the

Figure 1. The 4 main subgroups of protozoa are the flagellates (A), the apicomplexans (B), the sarcodines (C), and the ciliates (D). © 2016 ASRT.
pseudopods are similar to needles or spikes sticking out from the cell.³²

Ciliates are characterized by the presence of hair-like organelles, or *cilia*. The cilia are similar in structure to flagella, although they typically are shorter, move in a different pattern, and are present in much higher numbers. More than 3500 species have been described, and the estimated number of total species is approximately 30 000.³³ Most ciliates feed on smaller organisms and detritus swept into an oral groove by modified oral cilia.³⁴ Paramecia are typical, nontreating ciliates. The only ciliate known to be pathogenic to humans is the species *Balantidium coli*, which damages human intestinal mucosa. The occurrence of *B. coli* infection is rare; however, some infections have been due to close contact with pigs, and some waterborne transmissions have been documented.³⁵

**Giardia**

*Giardia* is a flagellate and the most widespread protozoan, causing more than 200 million infections worldwide.³⁶,³⁷ The *Giardia* life cycle begins when protozoan cysts enter the body via water that has come in contact with feces (see **Figure 2**). After ingestion, contact with stomach acid causes the cysts to break open. Excystation occurs, liberating 2 or 4 trophozoites, which are the actively feeding and multiplying stage of protozoans. The trophozoites tightly attach to the epithelial surface of the intestine via a ventral adhesive disk, or sucker, and the production of various parasitic products produces diarrhea. Under certain conditions in the jejunum, the trophozoites encyst, are passed in the stools, and immediately are infectious.³⁸

The *Giardia* cyst is highly resistant to environmental factors. It appears egg-shaped and measures 8 μm to 14 μm × 7 μm to 10 μm. When stained and observed under a microscope the nuclei become apparent. The immature *Giardia* cyst has 2 nuclei, whereas the mature cyst has 4 nuclei (see **Figure 3**). Genes allow *Giardia* to evade human immune response by shifting surface proteins, a characteristic not found in other parasites.³⁹

Giardiasis is one of the most frequently diagnosed intestinal parasitic diseases in the United States and among travelers who have chronic diarrhea.³⁹ Giardiasis is caused by an overgrowth of the parasite in the duodenum and jejunum. Signs and symptoms vary and last 1 to 2 weeks or longer. Acute symptoms include gas,
Cryptosporidium are recognized in the literature. Of these, 2 main species infect humans: C. parvum and C. hominis (formerly known as C. parvum anthroponotic genotype or genotype 1).

Cryptosporidiosis is an infection of the small intestine most often caused by C. parvum and C. hominis. The infection typically is acute and short term with the main symptom of self-limiting diarrhea. Common symptoms of cryptosporidiosis are similar to giardiasis and include lack of appetite and associated weight loss, stomach cramps or stomach aches, fever, nausea, and vomiting.

As with giardiasis, in immunocompromised individuals, such as people with AIDS, the symptoms can become particularly severe and fatal. Cryptosporidium is the organism most frequently isolated in HIV-positive patients with diarrheal disease. Patients with CD4+ cell counts below 200 are likely to experience prolonged symptoms.

People who travel to developing countries might be at greater risk for infection because of poorer water treatment and food sanitation systems, but crytosporidiosis occurs worldwide. Cryptosporidium parvum causes more human infections in Europe, especially in the United Kingdom, and C. hominis is prevalent in North and South America, Australia, and Africa.

In addition, Cryptosporidium can be found across the United States. More than 700,000 cases of cryptosporidiosis are estimated to occur each year in the United States, making Cryptosporidium a leading cause of waterborne disease.
Cyclospora

*Cyclospora cayetanensis* is another cyst-forming apicomplexan protozoan that causes disease in humans but was virtually unknown before 1980. *Cyclospora cayetanensis* is an obligate intracellular, coccidian protozoan pathogen. The spherical shaped oocysts can measure from 8 μm to 10 μm, compared with the 2 μm to 6 μm of those from *Cryptosporidium* (see Figure 6).85

The different stages in the *Cyclospora* life cycle are similar to those of *Cryptosporidium*; however, when *Cyclospora* oocysts are freshly passed in stools, the oocysts are not infective. Therefore, direct fecal-oral transmission does not occur. It normally takes days to weeks for *Cyclospora* oocysts to become infectious.84 As with *Cryptosporidium*, *Cyclospora* oocysts might survive for lengthy periods in the environment, given the marked seasonality of infection in areas where the disease is endemic.86

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**Figure 4.** Sporulated oocysts, containing 4 sporozoites, are excreted by the infected host through feces and possibly other routes such as respiratory secretions (1). Transmission of *Cryptosporidium parvum* and *Cryptosporidium hominis* occurs mainly through contact with contaminated drinking or recreational water. Occasionally, food sources, such as chicken salad, may serve as vehicles for transmission. Many outbreaks in the United States have occurred in water parks, community swimming pools, and day care centers. Zoonotic and anthropoanotic transmission of *C parvum* and anthropoanotic transmission of *C hominis* occur through exposure to infected animals or exposure to water contaminated by feces of infected animals (2). Following ingestion (and possibly inhalation) by a suitable host (3), excystation (a) occurs. The sporozoites are released and parasitize epithelial cells (b, c) of the gastrointestinal tract or other tissues such as the respiratory tract. In these cells, the parasites undergo asexual multiplication (schizogony or merogony) (d, e, f) and then sexual multiplication (gametogony), producing microgamonts (male; g) and macrogamonts (female; h). Upon fertilization of the macrogamonts by the microgametes (i), oocysts develop the sporulate in the infected host. Two different types of oocysts are produced: thick-walled, which commonly is excreted from the host (j), and thin-walled (k), which primarily is involved in autoinfection. Oocysts are infective upon excretion, thus permitting direct and immediate fecal-oral transmission. Reprinted from Parasites – *Cryptosporidium* (also known as “Crypto”). Centers for Disease Control and Prevention Web site. http://www.cdc.gov/parasites/crypto/pathogen.html. Updated February 20, 2015. Accessed May 16, 2016.

Cyclospora increasingly has been recognized as an enteric pathogen. Worldwide, cyclosporiasis is endemic in multiple areas, including Haiti, Asia, Puerto Rico, Europe, Indonesia, Pakistan, and India.17,59,63-66

Entamoeba

Entamoeba species are anaerobic parasitic protozoans that infect humans and other primates, causing amebiasis. Unlike Cryptosporidium and Giardia, Entamoeba species are not a common cause of traveler’s diarrhea.67

Entamoeba infection occurs when mature cysts are ingested and excystation takes place in the small intestine. Similar to Giardia, released trophozoites migrate to the large intestine. There, the trophozoites multiply and produce cysts. Both stages are passed in the infected person’s stool. Most often, the trophozoites remain in the lumen of the intestine; however, some might invade the intestinal mucosa, or enter the bloodstream and thereby manifest in extraintestinal sites, such as the liver, brain, and lungs. Cyst formation is triggered by the dehydration of gut contents in asymptomatic carriers. As with cysts from other protozoan pathogens, the Entamoeba cysts can survive for lengthy periods in the external environment whereas trophozoites passed in the stool will not persist outside the human body (see Figure 7).

In bright-field microscopy, Entamoeba cysts are spherical and usually measure 12 μm to 15 μm. A mature cyst has 4 nuclei whereas an immature cyst might contain only 1 to 3 nuclei. Trophozoites usually measure 15 μm to 20 μm (see Figure 8).67

Researchers have not established which species of Entamoeba actually causes amebiasis, because 3 species of intestinal amebae have identical morphologic characteristics: Entamoeba histolytica, Entamoeba dispar, and Entamoeba moshkovskii.68 Most symptomatic diseases are believed to be caused by E histolytica, which colonizes the human intestine, persisting as a commensal parasite, one that lives in or on a host without harming the host. This is similar to other amebae that are considered to be nonpathogenic and noninvasive. Entamoeba dispar and E moshkovskii generally are considered nonpathogenic. However, recently, some have reported that dysentery and extraintestinal disease are connected with E dispar and E moshkovskii, making their pathogenic potential unclear.18,69 Entamoeba histolytica and

Entamoeba histolytica will not experience significant symptoms. However, when Entamoeba trophozoites penetrate the intestinal wall of the infected person, they can produce amebic dysentery, a more severe form of amebiasis, with recurrent bloody stools and severe stomach cramping. Furthermore, if the ameba enters the bloodstream, it can reach the liver, heart, lungs, or brain, and more serious complications can arise, including liver abscesses, tissue destruction, and death.\(^70,71\)

**Naegleria**

The genus *Naegleria* includes more than 30 species of free-living amebae. Most of these microorganisms feed on bacteria and detritus.\(^72\) *Naegleria fowleri* lives in soil and bodies of warm fresh water; its prevalence and its severe toxicity to humans are highly concerning.

*Naegleria fowleri* goes through 3 stages: cyst, trophozoite, and a temporary flagellate stage.\(^73\) When

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in its trophozoite-ameboid form, *N. fowleri* is 15 µm to 30 µm long and 10 µm to 15 µm in diameter and produce broadly rounded lobopodia. Cysts are single walled, spherical, and 8 µm to 12 µm in diameter. Trophozoites transform to a flagellated form, which is smaller, with a pear shape and 2 flagellae at the end (see Figure 9).  

*Naegleria fowleri* causes an often fatal disease of the human central nervous system called *primary amebic meningoencephalitis* (PAM). Infection occurs when contaminated water enters the nose during water-related activities. Some cases have been caused by the use of nasal irrigation with Neti pots and sinus rinse bottles with contaminated tap water. PAM causes brain inflammation and destruction of brain tissue. Trophozoites infect a human host by penetrating the nasal mucosa and migrating to the brain via the olfactory nerves. The olfactory bulbs become inflamed and infection progresses quickly to the cerebral hemispheres, brain stem, and spinal cord.

Signs and symptoms of *Naegleria* infection can progress quickly and include fever, severe headaches, nausea, loss of balance, hallucinations, and seizures. Death usually occurs 3 to 14 days after exposure. PAM is difficult to detect because the disease progresses rapidly and diagnosis usually is made after death.

The first PAM infections were reported in 1965 in Australia. Tests on archived autopsy tissues determined that the first infections in the United States occurred in Virginia as early as 1937. In the United States in the past half century, 3 people out of 133 have survived this infection. PAM is relatively rare, and exposure to the pathogens might be more widespread because antibodies to *Naegleria* species are common in human sera.

**Balantidium**

*Balantidium coli* is the only ciliated protozoan parasite of humans. It is broadly distributed in warmer climates, where human infections most commonly occur. *Balantidium* is a rather large protozoan pathogen compared with *Cryptosporidium* and *Giardia*. The *Balantidium* life cycle includes 2 stages: a ciliated trophozoite and an environmentally resistant cyst. The trophozoites normally are oblong and spheroid, from 30 µm to 150 µm in length and 25 µm to 120 µm.

in width. Cysts, which most commonly are found in stools, are spheroid or ovoid, measuring 40 μm to 60 μm in diameter (see Figure 10).45

When the *B. coli* cyst is ingested, it passes through the host’s digestive system to the small intestine where trophozoites are released. Trophozoites colonize the lumen of the large intestine.83,84 There, the trophozoites flourish by feeding on the intestinal flora and replicate by binary fission. Dehydration in the lumen can cause trophozoites to die or undergo encystation and then exit the host via feces.83,84

*Balantidiosis* infections are rare in the United States. Generally, most individuals infected with *B. coli* experience no symptoms. Immune-compromised individuals are the most likely to experience more severe signs and symptoms, including persistent diarrhea, dysentery, abdominal pain, weight loss, nausea, and vomiting. When left untreated, perforation of the colon can occur. Severe *B. coli* infections can resemble amebiasis because *B. coli* can penetrate the mucosa, resulting in ulceration similar to *E. histolytica*, but perforation is more common.

Metastatic lesions do not occur; extraintestinal disease has been reported but is rare.42

**Infectious Dose**

Several factors contribute to protozoan parasites’ success. Large quantities (thousands to millions) of cysts or oocysts are excreted into the environment when they exit the host. The cysts are environmentally resilient and able to survive for many months in cold, temperate, and moist conditions. Perhaps most importantly, infection in a new host can be initiated by a very small number of parasites. Presumably, a single parasite can cause infection; however, several studies have estimated the median infective dose, or the amount of pathogenic microorganisms that produce demonstrable infection in 50% of test subjects (ID<sub>50</sub>). For *G. duodenalis*, the ID<sub>50</sub> has been estimated between 25 and 100 cysts.85,86 Similarly, the ID<sub>50</sub> for *Cryptosporidium* ranges from 10 to 132 oocysts.87,88 These doses are quite low compared with infective dose estimates (> 100 000) for *Escherichia coli*, *Bacillus anthracis* (anthrax), and *Salmonella*.89 The ID<sub>50</sub> for *Cyclospora cayetanensis* infection in a wide variety of animal models and humans has been difficult to obtain. The infectious dose is relatively unknown but is thought to be low.90-92 No ID<sub>50</sub> estimates for amebae have been reported.

**Routes of Transmission**

Exposure via drinking water and recreational water are the most common modes of transmission for various protozoa. To some degree, the microbial quality of drinking water is regulated in the United States by the Total Coliform Rule and the Surface Water Treatment Rule.93,94 In 1987, *Cryptosporidium* was listed as a contaminant of concern under the Safe Drinking Water Act. Because *Cryptosporidium* and *Giardia* can survive wastewater treatment processes, strict regulations in the United States specifically address *Cryptosporidium* in potable water supplies.95 The Long Term 2 Enhanced Surface Water Treatment Rule from the U.S. Environmental Protection Agency requires drinking water source monitoring for *Cryptosporidium* oocysts.96 The Interim Enhanced Surface Water Treatment Rule requires that 99.9% of *Cryptosporidium* oocysts and *Giardia* cysts be removed from drinking water.97 However, no water...
quality regulations exist for *Naegleria* in public and private systems, and water testing for this microorganism is extremely limited.

The 2 most frequent means to kill microorganisms in the public water supply are oxidation with chemicals, such as ozone, chlorine, or chlorine dioxide, and treatment with ultraviolet radiation. Various protozoa reside in unchlorinated water supplies and can propagate in water treatment or distribution systems and in tap water installations in buildings.\(^{98-101}\) For household protection, filtration systems using activated carbon, ultraviolet light, reverse osmosis, and other methods are the most effective way to eliminate these pathogens.\(^{102}\)

Waterborne illnesses also can be acquired from lakes, ponds, rivers, oceans, swimming pools, and hot tubs. Every year hundreds of people in the United States acquire waterborne illnesses even though the country has some of the cleanest recreational water sources and the best disease prevention programs.\(^{103-105}\)

*Cryptosporidium* is responsible for half of reported waterborne disease outbreaks associated with public swimming pools.\(^{106}\) The pathogen’s continual excretion for weeks after symptoms have subsided and its resistance to chlorine has led the CDC and the American Academy of Pediatrics to recommend that infected people refrain from swimming until 2 weeks after symptoms resolve.\(^{107}\) One person with pathogen-associated diarrhea can contaminate the water in a large swimming pool or pond in a short time. Swallowing even a small amount of contaminated recreational water can cause illness. *Cryptosporidium*, which can stay alive for days even in well-maintained pools, has become the leading cause of swimming pool–related outbreaks of diarrheal illness. Although microbes such as *Cryptosporidium* and *Giardia* are tolerant to chlorine, most are not. Therefore, keeping chlorine at recommended levels is necessary for maintaining a sanitary swimming pool. A 2010 study showed that codes were not always followed, and more than 10% of public pool inspections resulted in immediate pool closure because of serious code violations, such as improper chlorine levels.\(^{108}\) From 2004 to 2008, reported cryptosporidiosis cases increased more than 200%.\(^{109}\)

Chlorine, although a good antimicrobial agent, does not kill all microbes instantly; several are highly tolerant to chlorine and were not known to cause human disease until recently. Oocysts from *Toxoplasma gondii*, which is related to *Cryptosporidium*, are also highly resistant to chemical and physical disinfection. *Naegleria* amebae are moderately susceptible to chlorine, and its use is recommended for ridding water of the amebae. However, chlorine disinfection is ineffective at killing *Naegleria* amebae in biofilms that coat drinking water pipes.\(^{110}\) *Naegleria* does not survive in sea water.\(^{111}\)

**Diagnostic Approaches**

Diagnosis of cryptosporidiosis and giardiasis is made through examination of stool samples. Because *Giardia* cysts and *Cryptosporidium* oocysts can be excreted intermittently, collecting 3 stool specimens on separate days increases test sensitivity.\(^{112}\) Microscopy methods such as acid-fast staining, direct fluorescent antibody testing, and enzyme immunoassays often are used to detect *Cryptosporidium* species antigens. However, microbe staining and concentration methods are not always sufficient to identify some parasites because of the variability of concentration in the stool. Therefore, physicians use rapid immune-chromatographic cartridge assays and fecal immunoassays for clinical diagnosis.\(^{113}\) These tests have shortcomings, and methods such as microscopy require evaluation by a skilled specialist. Even then, differentiating between the species and subtypes is difficult. Molecular methods such as polymerase chain reaction or DNA sequencing, although not routinely performed at most clinical laboratories, can be used to identify the subtypes of certain parasites.

*Cyclospora* oocysts are passed in stools in low to moderate numbers. *Cyclospora* identification might be difficult when symptomatic individuals do not shed enough oocysts in their stool to be detected by laboratory examinations and submit several specimens collected on different days. *Cyclospora* oocysts are recognized easily using conventional microscopy to establish a proper clinical diagnosis of cyclosporiasis. *Cyclospora* oocysts are autofluorescent. Fluorescent microscopy using a filter with a range of 340 nm to 380 nm causes the oocysts to glow a bright, pale blue. Acid-fast staining also can be used to make the oocysts more visible under the microscope. When stool containing *Cyclospora* is viewed under an ultraviolet fluorescent microscope, the parasite appears
blue or green against a black background. Molecular diagnostic methods also can be used to detect the parasite's DNA in the stool.\textsuperscript{114}

Microscopy does not differentiate \textit{E histolytica} from its counterparts, \textit{E dispar} and \textit{E moshkovskii}, because the species are morphologically identical. Rapid immunochromatographic cartridge assay is available to detect antigens of \textit{E histolytica}, \textit{E dispar}, and \textit{E moshkovskii} but does not distinguish between them. However, molecular techniques can differentiate the species.\textsuperscript{115}

Medical imaging can be used to localize abnormalities and rule out the involvement of some structures before performing capsule endoscopy, in which the patient ingests a small capsule containing a wireless camera. It is as sensitive or more sensitive than a radiological examination for detecting subtle mucosal abnormalities.\textsuperscript{116,117} The camera transmits high-quality images of the mucosa along the entire length of the small bowel.\textsuperscript{118} Leighton et al found capsule endoscopy to be superior to small bowel follow-through (SBFT) and equivalent to ileocolonoscopy for delineating small-bowel inflammation.\textsuperscript{119} CT enterography and capsule endoscopy often complement each other, each providing information that the other cannot.\textsuperscript{120} The disadvantages of capsule endoscopy include capsule retention, false positives, difficulty in lesion localization, and failure to reach the cecum.\textsuperscript{121}

\textbf{Medical Imaging}

Medical imaging is not a first-line diagnostic approach for waterborne pathogen–related diseases. Most patients do not seek medical attention and, in those who do, diagnoses often can be made from the patients' history, physical examination, stool studies, and if needed, endoscopy. Imaging usually becomes necessary in cases involving immunocompromised patients and AIDS–related diseases in which complicated opportunistic infections can threaten a patient's life.\textsuperscript{122,123} In certain clinical circumstances, abdominal pain associated with waterborne disease is evaluated with medical imaging.\textsuperscript{124}

\textbf{Small Bowel Imaging}

Effective imaging of the small bowel depends on choosing the appropriate investigational strategy to answer clinical questions. The small bowel can react morphologically to pathology in various ways, including dilatation, stricture formation, and wall thickening, and identifying these abnormalities can lead to a specific diagnosis.\textsuperscript{125} To visualize the small bowel, barium fluoroscopy, such as SBFT, magnetic resonance (MR) imaging, computed tomography (CT) enterography or CT enteroclysis, ultrasonography, and endoscopy or capsule endoscopy might be used. The choice of modality often is determined by local availability and expertise, and technical details of these examinations might vary, which could affect accuracy.

Historically, small bowel examinations have been performed using barium fluoroscopy, generally SBFT.\textsuperscript{126,127} SBFT is readily available, consistent, and easy to perform. The procedure often is performed with a 40% to 50% weight per volume barium suspension, a density low enough to permit display of abnormalities within the barium-filled small-bowel lumen. Barium studies on patients with gastrointestinal disease might reveal thickened, irregular nodular folds, predominantly in the duodenum and jejunum.\textsuperscript{128} The uniformly thickened folds have a relatively parallel configuration, producing a “stack of coins” appearance (see Figure 11).

Although SBFT commonly has been used for small bowel evaluation, it has a low diagnostic yield. Abdominal radiography and CT scanning, although often nonspecific, might reveal distended loops of bowel, air-fluid levels, and disrupted bowel motility. Conventional CT imaging might reveal mesenteric lymphadenopathy, bowel wall dilatation and thickening, thickening of either or both of the duodenal and jejunal folds, pneumatosis, and dilution and flocculation of oral contrast. Although CT is sensitive and specific for certain diagnoses, small-bowel thickening is a nonspecific finding using conventional CT with a broad differential diagnosis and positive oral contrast that obscures the bowel wall.\textsuperscript{129}

\textbf{Computed Tomography}

Recent advancements in speed and resolution have made CT a powerful tool for assessing inflammatory diseases of the gastrointestinal tract, especially extraenteric complications such as abscess, fistula, and obstruction. The advantages of multidetector CT
The lesions had ring enhancement and were located predominantly in the right lobe of the liver, suggestive of and later confirmed to be abscesses. In another patient with abdominal pain, an abdominal contrast-enhanced CT scan showed an irregular mass in the cecum and an irregular and nodular inhomogenously enhancing mass in the hepatic flexure of colon that warranted a colonoscopy. Entamoeba infection was diagnosed and treated with antibiotics.

CT enterography, CT enteroclysis, and capsule endoscopy are reforming small bowel evaluation. CT enterography uses a combination of oral contrast and intravenous contrast. CT enterography has gained widespread acceptance as a method for evaluating small bowel inflammation and masses. The examination can delineate active inflammatory disease while allowing a global overview of bowel pathology not provided by other techniques. CT enterography also displays the entire small bowel wall and extraenteric impediments of small bowel disease and is superior to SBFT for diagnosis of inflammatory bowel disease with comparative radiation doses and better suitability for children.

CT enteroclysis involves using fluoroscopy to introduce an enteroclysis tube to administer the contrast followed by abdominal CT. The procedure might be uncomfortable for some individuals, requires additional expertise, and is more time consuming than some other examinations. It also provides similar information to CT enterography and might provide no advantage if the patient can ingest the oral contrast in sufficient quantities. CT enterography and CT enteroclysis improve imaging of the small bowel mucosa and wall compared with conventional CT and fluoroscopic methods by distending the small bowel with a negative contrast agent. Negative contrast better displays the small bowel lumen and the enhancing mucosa than does higher-density contrast. When it can be tolerated, CT enterography is preferred because it is less invasive.

Magnetic Resonance

MR enterography and MR enteroclysis are potential alternative imaging techniques if CT is not available. MR imaging provides improved spatial and temporal resolution and reduced motion artifacts. Masselli et al found MR enterography to be more accurate than CT.
enterography for detecting small-bowel diseases. Preference of MR enterography over CT enterography might be based on available resources and public policies, as well as lack of radiation exposure.

**Ultrasonography**

When directed by initial symptoms and as in the case of cryptosporidiosis, ultrasonography or CT scans might reveal thickened walls of enlarged gallbladders as well as dilated intrahepatic and extrahepatic biliary ducts. Ultrasonography using high-frequency (5 MHz-17 MHz) linear array transducers provides excellent spatial resolution of the intestinal wall. In addition, amebic liver abscess can be evaluated with ultrasonography, which has been shown to be as sensitive as nuclear medicine scans for determining the number, size, and location of lesions. Ultrasonography also can guide therapeutic drainage (ie, guided percutaneous liver abscess drainage).

**Follow-up Imaging**

Ultrasonography, chest radiography, and abdominal CT scans are common choices for follow-up evaluations. Ultrasonography is practical for evaluating children and adolescents because no ionizing radiation is used. Cavailloles et al used positron emission tomography-computed tomography with fludeoxyglucose F 18 (FDG PET-CT) to diagnose secondary hepatic lesions associated with amebic abscess.

**Severe Disease**

More serious diseases, such as PAM, can be evaluated with CT and MR imaging; however, because PAM progresses so rapidly, these examinations usually are performed postmortem. Although brain lesions can be diagnosed with either technology, MR is more sensitive. For example, MR scans can show infection and the range and depth of necrosis in soft tissue. Occasionally, additional modalities are necessary, such as MR spectroscopy, single photon emission computed tomography (SPECT), and magnetic transfer contrast imaging, to aid examination and diagnosis.

PAM often has nonspecific imaging features and is difficult to distinguish from bacterial and amebic meningoencephalitis (granulomatous amebic encephalitis [GAE]). One study evaluated initial CT and MR imaging of the brain in 5 proved cases of amebic meningoencephalitis (PAM and GAE). CT scans revealed ill-defined hypoattenuated lesions in the left temporo-parietal area of the brain. MR imaging of patients with GAE revealed multifocal involvement whereas contrast-enhanced CT scans of the brain showed an enhancing cortical-based lesion in the left parietal lobe with extensive perilesional edema. The subject with PAM showed evidence of obliteration of the cisterns with enhancing basilar exudates and infarction of the right basal ganglia. In a separate case of PAM, a repeat CT scan showed a lesion in the right frontal lobe and diffuse cerebral edema (see Figure 12).

**Treatment**

Most people who have healthy immune systems recover from cryptosporidiosis and giardiasis without medical intervention. Dehydration and diarrhea can be treated by drinking plenty of fluids. However, people who are in poor health or have weakened immune systems are at higher risk for a more difficult and prolonged illness. To prevent dehydration, over-the-counter medications for diarrhea, such as loperamide (Imodium), might relieve symptoms, but a health care provider should be consulted before use. Young children and pregnant women likely are more susceptible to dehydration. Rapid loss of fluids can be especially life threatening to infants.

Antibiotics are available for cryptosporidiosis and giardiasis. Nitazoxanide (Alinia) is an FDA-approved antiprotozoal medication for treatment of diarrhea caused by *C parvum* and *G lamblia* in people with healthy immune systems and is available by prescription. However, its effectiveness in immunosuppressed individuals is unclear. Patients who have HIV and suspect they have cryptosporidiosis should work with their health care provider to identify alternatives. For patients with AIDS, antiretroviral therapy that improves immune status also decreases or eliminates symptoms of cryptosporidiosis. However, even if symptoms disappear, cryptosporidiosis often is incurable and symptoms can return if immune status worsens.

The most common treatment for giardiasis is the antibiotic metronidazole (Flagyl), which has an efficacy rate of 75% to 100%. Metronidazole occasionally causes adverse gastrointestinal effects such as nausea.
and headache. Nevertheless, metronidazole is not approved by the FDA for treatment of giardiasis in the United States. Tinidazole has replaced furazolidone as the FDA-approved drug for treatment of the condition and is highly effective. It can be given as a single dose and is well tolerated. Previously used drugs, such as furazolidone and quinacrine hydrochloride, are considered less effective and are no longer sold in U.S. markets. Sometimes, treatment fails to eradicate Giardia. In these cases, physicians might prescribe combination therapy, select an alternative medication, or increase dose and duration of use. The only CDC-recommended treatment is sulfamethoxazole/trimethoprim. In the past, individuals with cyclosporiasis experienced successful outcomes with a regimen of co-trimoxazole (160 mg trimethoprim and 800 mg sulfamethoxazole). No other substantially effective medication regimen has been identified.

Most cases of PAM caused by Naegleria infection have been fatal. However, in a few examples, recovery occurred following antimicrobial therapy. For example, a 10-year-old boy developed PAM after swimming and recovered after he was treated with intravenous dexamethasone, amphotericin B, and fluconazole, and oral rifampicin. Miltefosine, an investigational breast cancer drug, reportedly helped a young girl with PAM recover and has shown promise in combination with other drugs. An in vitro study of miltefosine has shown ameba-killing activity against free-living amebae, including Naegleria. Other drug regimens have worked to some extent but have not led to optimal recovery rates. The CDC recommends a combination of drugs based on the treatment regimens used in survivors of PAM. Trials evaluating the use of vaccines against these infections have been implemented. As of summer 2016, no vaccines exist to prevent Cryptosporidium, Cyclospora, Giardia, and Naegleria infections.

**Waterborne Pathogen Monitoring**

Waterborne pathogens and related diseases are a serious worldwide public health concern because of the morbidity and mortality they cause and the high costs involved in prevention and treatment. Proper assessment of pathogen presence in water and water quality monitoring initiatives are key factors in making decisions about water distribution and infrastructure as well as choosing the best water treatment for the prevention of waterborne disease outbreaks.

The goal of microbial source tracking is to provide information about the origin of fecal pollution. In the United States, many public health departments use various fecal indicator bacteria methods to estimate levels of fecal coliforms, such as E coli. Limitations of these indicator methods are that they do not provide information about the hosts (ie, whether they are human or another mammal), and the methods do not correlate well with the presence or absence of protozoan pathogens.

In the United States, water facilities and laboratories must use certain methods to monitor pathogenic protozoa. These methods have not been updated for more than a decade, and they employ microscopy instead of proven molecular methods. Nevertheless, monitoring systems have been standardized for Cryptosporidium and Giardia monitoring. No standardized filter or filtration method has been developed for Cyclospora and Naegleria. Methods using cartridge filters are not cost effective (~$60-$100 per filter), so efforts have been directed at detecting the presence of pathogens using filter-feeding bivalves and biofilms.
The turbidity of water also is a problem. The dirtier the water, the more difficult it is to separate target organisms from nontarget organic and inorganic materials. Techniques such as immunomagnetic separation have been developed to fix these problems, and other diagnostic tools have been developed that can detect the viable but nonculturable microorganisms.

Conclusion

Waterborne pathogens have a major effect on human health, socioeconomics, and agricultural productivity, and we have little to moderate defense against them. However, 2014 WHO statistics showed a 15% increase in the number of people (more than 2.6 billion people) who had access to an improved drinking-water source from 1990 to 2012. Despite continued efforts to maintain water safety, waterborne pathogen-related outbreaks still are reported globally. Overwhelming water demand; increasing resistance of pathogens to disinfection; and increasing population densities and the resulting growth in agricultural, industrial, and human waste discharge increasingly make clean and safe water a valuable resource.

Joseph Anthony Moss, MS, is a research associate for the Center for Environmental Diagnostics and Bioremediation with the University of West Florida in Pensacola, Florida. A molecular biologist and author of many research articles and literature reviews involving molecular biology and microbiology, Moss also works to develop cost-effective methods to detect waterborne pathogens, studies microbial communities in the Pacific Ocean and Gulf of Mexico, and teaches fundamentals about microorganisms in the production of beer.

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References


Waterborne Pathogens: The Protozoans


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110. Naegleria fowleri—primary amebic meningoencephalitis


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Read the preceding Directed Reading and choose the answer that is **most correct** based on the article.

1. The World Health Organization estimated that in 2014, ______ people did not have access to safe drinking water.
   a. 1.8 million
   b. 18 million
   c. 1.8 billion
   d. 18 billion

2. Which pathogens cause **most** reported gastrointestinal disease?
   1. Cryptosporidium
   2. Entamoeba
   3. Giardia
   a. 1 and 2
   b. 1 and 3
   c. 2 and 3
   d. 1, 2, and 3

3. ______ allow Giardia to evade human response by shifting surface proteins, a characteristic not found in other parasites.
   a. Genes
   b. Schistosomes
   c. Triungulinids
   d. Transcription factors

4. A Cryptosporidium oocyst can survive in the environment under harsh conditions for **more** than ______ months.
   a. 2
   b. 3
   c. 6
   d. 9

5. Which microorganism causes an often fatal disease of the human central nervous system called primary amebic meningoencephalitis (PAM)?
   a. Cryptosporidium parvum
   b. Cyclospora cayetanensis
   c. Giardia duodenalis
   d. Naegleria fowleri

**continued on next page**
6. Amoebic liver abscesses are frequent extra-intestinal manifestations found most often in immunocompromised patients who have which type of infection?
   a. *Entamoeba*
   b. *Giardia*
   c. *Naegleria fowleri*
   d. *Cyclospora*

7. Which of the following are true about computed tomography (CT) enterography?
   1. It can delineate active inflammatory disease while allowing a global overview of bowel pathology not provided by other examinations.
   2. The scans can display the entire small bowel wall and extraenteric impediments of small bowel disease.
   3. The examination is superior to small bowel follow-through for diagnosis of inflammatory bowel disease.

   a. 1 and 2
   b. 1 and 3
   c. 2 and 3
   d. 1, 2, and 3

8. According to the article, when directed by initial symptoms and as in the case of cryptosporidiosis, ultrasonography or CT scans might reveal which of the following signs?
   a. thickened walls of enlarged gallbladders
   b. stenosed intrahepatic and extrahepatic biliary ducts
   c. thinned walls of enlarged gallbladders
   d. a “stack of coins” appearance

9. Which of the following can show infection and the range and depth of necrosis in the soft tissue of patients with PAM?
   a. ultrasonography
   b. CT
   c. magnetic resonance imaging
   d. capsule endoscopy

10. Although costly, vaccines are available to prevent *Cryptosporidium, Cyclospora, Giardia,* and *Naegleria* infections.
    a. true
    b. false