282 Cryptosporidiosis (*Cryptosporidium* Species)

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SHORT VIEW SUMMARY

Definition
- Cryptosporidiosis is caused by ingestion of oocysts of *Cryptosporidium* species.

Epidemiology
- Cryptosporidiosis has a global distribution with a higher prevalence in resource-poor countries.
- Cryptosporidiosis is a major cause of prolonged diarrhea and malnutrition in children in resource-poor countries.
- Cryptosporidiosis is a major cause of diarrhea in adults infected with human immunodeficiency virus (HIV).
- Cryptosporidiosis is associated with person-to-person and waterborne transmission in wealthy countries.
- The major species, *Cryptosporidium hominis,* primarily infects humans.
- Zoonotic species, including *Cryptosporidium parvum,* are also common in humans.

Microbiology
- *Cryptosporidium* species are apicomplexan protozoan parasites.

Clinical Manifestations
- Most patients present with diarrhea that is frequently prolonged.

Diagnosis
- Diagnosis depends on demonstration of the organism in stool through antigen detection, nucleic acid amplification, or microscopy with acid-fast or fluorescent stains.

Management
- For immunocompromised hosts, reversal of immune defects is critical (e.g., treating underlying HIV with antiretroviral therapy or decreased immunosuppression in transplant recipients).
- Nitazoxanide alone is effective in immunocompetent hosts but not alone in severely immunocompromised patients.

Prevention
- Water treatment and hand hygiene are key measures to prevent infection.

Protozoan parasites of the genus *Cryptosporidium* were first identified in the stomach of mice in 1907.[1] The species name *Cryptosporidium parvum* was proposed in 1912 to describe parasites identified in murine intestines.[2] Although *Cryptosporidium* was linked to gastrointestinal disease in turkeys in 1955 and to bovine diarrhea in 1971, the first human cases were described only in 1976.[3–6] Only a handful of cases had been reported before 1982. In the early 1980s, large numbers of cases were noted to be associated with the emerging epidemic of acquired immunodeficiency syndrome (AIDS).[7] Soon studies identified cases among animal handlers and children.[8] Shortly thereafter, *Cryptosporidium* was associated with waterborne outbreaks of diarrhea, including an outbreak in Milwaukee, Wisconsin in 1993 that affected an estimated 403,000 persons.[9] Studies have now demonstrated that *Cryptosporidium* is an important cause of diarrhea in normal hosts worldwide and one of the main causes of childhood diarrhea in resource-poor countries, including causing prolonged diarrhea and malnutrition, and of chronic diarrhea in immunocompromised hosts, including patients with AIDS. Cryptosporidiosis is now recognized as a major cause of childhood diarrhea morbidity and mortality.[10–12]

THE PARASITES

The genus *Cryptosporidium* consists of a group of protozoan parasites within the phylum Apicomplexa, which also includes *Plasmodium* species. *Cryptosporidium* has been reclassified from the subclass Coccidiasina (coccidia, along with *Toxoplasma, Cyclospora,* and *Cystoisospora*) into the class Gregarinomorphea, subclass Cryptogregaria.[13–15] The genomes of a number of *Cryptosporidium* species have been sequenced, including *Cryptosporidium hominis*, *C. parvum*, an anthroponotic strain of *C. parvum* subtype IIc, and others.[16–23] Compared with other apicomplexan parasites, the genomes are relatively compact (approximately 9.1 Mb), with the loss of approximately 1400 genes, compared with the *Plasmodium* parasites. Many of these gene deletions may be due to loss of the mitochondria and apicoplast, organelles found in most other apicomplexans but not found in *Cryptosporidium* spp. *Cryptosporidium* spp. also lack the genes for variable surface proteins contained in the *Plasmodium falciparum* genome (e.g., *var*, *rif*, and *stevor* genes). The metabolic pathways are also simplified (e.g., no Krebs cycle), but a number of transporters are present to scavenge molecules from the host. Important metabolic pathways do exist, however, such as fatty acyl-coenzyme A synthetase, and their inhibition in a mouse model reduced parasite oocyst production.[24]

Species were initially named based on the host species. In the late 20th century, human isolates were thought to belong to a single species, *C. parvum*. Molecular studies subsequently demonstrated that parasites previously termed *C. parvum* include a number of genotypes and occult species.[15,25] As of 2018, there were at least 35 named *Cryptosporidium* spp. thought to be valid, based on host specificity, morphology, and molecular biology studies, and numerous other genotypes that may emerge as separate species. Among the isolates speciated as *C. parvum,* however, there is also a subtype, IIc, that mainly infects people and shares sequence homology in some regions with *C. hominis* instead of other *C. parvum* isolates.[18,25,26] Molecular biology studies have demonstrated that humans can also be infected with *Cryptosporidium meleagridis, Cryptosporidium cuniculus, Cryptosporidium ubiquitum, Cryptosporidium viatorum, Cryptosporidium canis, Cryptosporidium felis, Cryptosporidium muris,* and others.[27–29] No specific clinical characteristics of rare species have been reported, but volunteer studies have demonstrated mild diarrhea with *C. meleagridis* and *C. muris.*[30,31] *C. meleagridis,* formerly thought to mainly infect birds, has been identified in most large series and appears to cause approximately 1% of human cryptosporidiosis overall and more in some series from Asia.[29,32] Other species are either rarely noted to cause human infection or have been noted to infect only reptiles, fish, birds, or nonhuman mammals.[31]

Cryptosporidium spp. can complete their entire life cycle within a single host, including both asexual (merogony) and sexual (sporogony) reproductive cycles (Fig. 282.1).[10,33] In the stomach and upper intestines, the oocysts are activated, producing serine and cysteine proteases and aminopeptidases, which allow the organisms to excyst, releasing four infective sporozoites.[33,34] Contact with the sialylated carbohydrate surface of the epithelial cells may be an important trigger for excystation.[35]

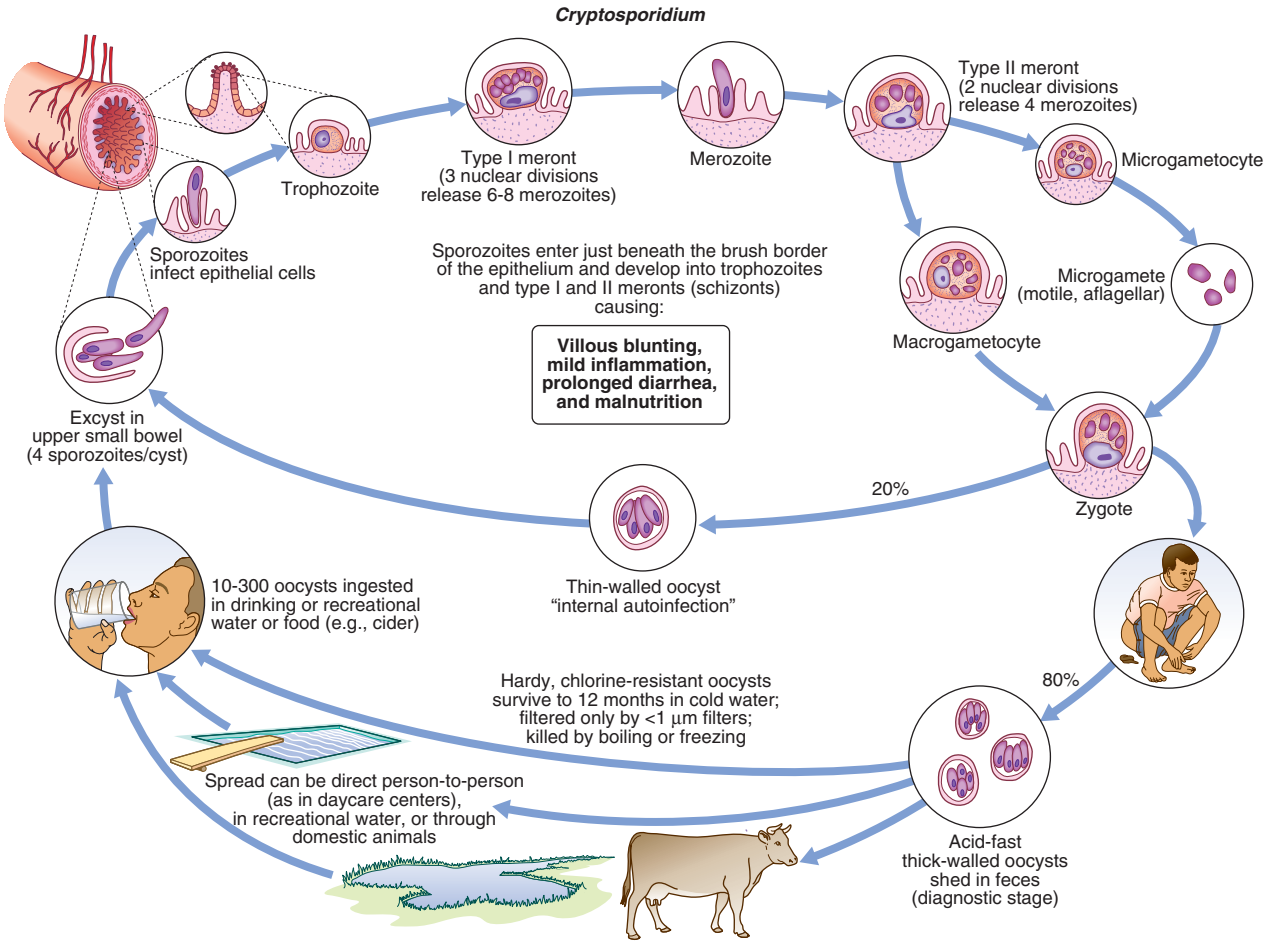

FIG. 282.1 Life cycle of *Cryptosporidium*. Oocysts are excreted in the feces. After ingestion, the sporozoites are released from the oocysts and attach to and invade intestinal epithelial cells. The cells engulf the parasites into a parasitophorous vacuole, where they enlarge to form the trophozoites; undergo asexual multiplication, forming type I meronts; and release the motile merozoites. The merozoites reattach to epithelial cells. They may continue the asexual cycle or may undergo sexual multiplication, producing type II meronts. The type II meronts differentiate into microgamonts and macrogamonts. The microgametes fertilize the macrogametes to form the zygote. The zygotes develop into the oocysts. Two different types of oocysts are produced: the thick-walled oocyst, which is commonly excreted from the host, and the thin-walled oocyst, which is primarily involved in autoinfection. Oocysts are infective at excretion, thus permitting direct and immediate fecal-oral transmission. *(From Lima AM, Samie A, Guerrant RL. Cryptosporidiosis. In: Guerrant RL, Walker DH, Weller PF.* Tropical Infectious Diseases. *3rd ed. Philadelphia: Saunders; 2011.)*

Other factors in excystation may include temperature, stomach acid, bicarbonate pancreatic enzymes, and bile.[36] Each sporozoite contains an apical complex with specialized organelles involved in invasion, including rhoptries, micronemes, and dense granules.[36] The motile sporozoites bind to receptors on the surface of the intestinal epithelial cells. Several parasite ligands (including p30, the galactose-*N*-acetylgalactosamine lectin; p23, the 1300-kDa circumsporozoite-like antigen; gp900, the thrombospondin-related adhesive protein of *Cryptosporidium*-1, Cpgp40/15, and CP47) have been implicated in parasite attachment to the intestinal epithelium.[33,34] The parasites then induce actin polymerization and protrusion of the intestinal epithelial cell membrane, which is mediated by tyrosine kinase growth factor receptor (TKGFR), phosphatidylinositol 3 (PI3) kinase, and CDC42.[37–39] The membrane surrounds the sporozoite and fuses to form the parasitophorous vacuole, which remains in the microvillus layer on the surface of the epithelium.[40] A band of dense cytoskeletal elements separates the parasite from the host cytoplasm.[40] This band prevents free flow of materials between parasite and the host cell cytoplasm.[41] It also contains an adenosine triphosphate–binding cassette, which likely functions as an efflux pump, contributing to the resistance of the organisms to chemotherapy.[42] Inside the parasitophorous vacuole, the parasites undergo asexual reproduction (merogony). They enlarge into trophozoite forms and divide to form type I meronts, which mature and rupture to release the motile merozoites. The merozoites bind to receptors on the epithelial cells and are engulfed by the cells. They then either repeat the process of merogony or undergo sexual differentiation. In that case, the merozoites differentiate into the microgamonts and macrogamonts. The microgamont releases the microgametes, which penetrate the cells infected with a macrogamont. The macrogamont and microgametocytes fuse to form the zygote form, which then undergoes meiosis to form the oocyst, containing four sporozoites. Two morphologic forms of the oocyst have been described. Thin-walled oocysts are thought to excyst within the same host in a process of self-infection. The thick-walled oocysts are shed into the environment.

EPIDEMIOLOGY

Several factors define the epidemiology of cryptosporidiosis.[33,43–46] First, the oocysts are infectious when shed. Thus, parasites are readily transmitted directly from person to person. Second, although *Cryptosporidium* does not multiply outside of the host, the infectious dose is low, facilitating transmission from sources with low-grade contamination, such as recreational water. Third, the oocyst stage can survive for prolonged periods in the environment and resists disinfection, including chlorination.[47,48] Its small size and resistance to chlorination facilitate waterborne transmission. Fourth, the host immune response limits the duration and severity of infection such that disease is more commonly recognized in children (preimmune) or in compromised hosts, especially patients with AIDS. Although some genotypes have important animal reservoirs, molecular studies demonstrate that most human infection is caused by species and subtypes that mainly affect humans. Still, transmission of some species (e.g., *C. parvum*) is associated with animal contact, rural

areas, and exposure to surface water.[44] In contrast to zoonotic species, *C. hominis* is more common in urban settings and is associated with higher population density (especially the number of children).[44] In some studies, household income is not protective against cryptosporidiosis and may pose an increased risk of *C. hominis* infection.[49]

Cryptosporidium parasites have been found in every region of the world except Antarctica. Infection is generally more common in warm or moist months.[50–52] For example, cases in the United States peak in late summer and early autumn.[53] Studies from England found that *C. parvum* peaks in the spring, whereas *C. hominis* peaks in autumn.[44] The peak that occurs in spring is thought to be associated with runoff from pastures, and the autumn peak follows the season for swimming in recreational water. Most studies on the prevalence of infection have relied on detection of oocysts in fecal specimens submitted for parasitologic examination. Fewer than 3800 cases per year were reported in the United States from 1995 through 2002, but the number of reported cases increased to more than 7600 per year after then, without other evidence suggesting more illness.[53,54] This increase coincided with the marketing of nitazoxanide and was thought to be the result of increased awareness and improved diagnosis. Inconsistencies exist in these data, perhaps driven by changes in diagnostic criteria. By contrast, estimates based on antibody prevalence suggest that more than 750,000 persons in the United States are affected each year.[55] The main reason for this difference stems from both insensitivity and underuse of diagnostic tests for *Cryptosporidium*.[56–58] With improving diagnostic techniques, more cases are also being identified in high-income countries. Similarly, studies from England and the Netherlands showed that use of polymerase chain reaction (PCR) assays led to a doubling of the number of *Cryptosporidium* cases identified.[59,60]

The prevalence of cryptosporidiosis in low- and middle-income countries has been characterized in three large multicenter studies of diarrhea in resource-poor countries; these studies used sensitive techniques to study all of the major pathogens.[11,50,61–63] The Global Enteric Multicenter Study (GEMS) was a case-control study of moderate-to-severe diarrhea conducted at seven centers in sub-Saharan Africa and South Asia. *Cryptosporidium* was second to rotavirus as a cause of diarrhea in children younger than 2 and was associated with an increased fatality rate in infants and toddlers.[11] Based on these data and a subsequent study of milder diarrhea, the authors estimated that 7.6 million children in South Asia and sub-Saharan Africa develop diarrhea due to cryptosporidiosis annually and that those infections are associated with over 200,000 deaths.[61] The Malnutrition and Enteric Disease Study prospectively studied birth cohorts from India, Bangladesh, Nepal, Tanzania, South Africa, Brazil, and Peru. *Cryptosporidium* was among the top four causes of diarrheal disease.[50,64] Among 1486 individuals for whom full data through 2 years were available, 962 (65%) developed a *Cryptosporidium* infection. The World Health Organization (WHO) Global Rotavirus Surveillance Network collects stool specimens from children hospitalized with acute watery diarrhea from 178 sentinel surveillance sites in 60 countries. A recent study tested 878 stool samples from children with watery diarrhea in sub-Saharan Africa, India, Myanmar, Philippines, and Brazil for enteric pathogens by PCR card.[62] The fraction of diarrheal episodes attributable to *Cryptosporidium* was 5.8%, with slightly higher rates in children younger than 2. Rates were less than for rotavirus but similar to those of norovirus.[62] A recent report on global burden of diarrheal disease estimated that there are 48,000 child deaths each year from cryptosporidiosis and more than 4.2 million disability-adjusted life-years lost.[65]

Prospective studies of birth cohorts in South Asia have noted that virtually all children are infected by age 2.[66,67] Summary data from published and unpublished sources in India suggest that that country may see 3.9 to 7.1 million diarrheal episodes, 66,400 to 249,000 hospitalizations, and 5800 to 14,600 deaths each year in children younger than 2 years.[68] Older studies from sub-Saharan Africa have identified *Cryptosporidium* in 7.5% to 22.2% of cases with use of microscopy.[69,70] However, studies using PCR or antigen-detection methods have documented *Cryptosporidium* in up to 30% of acute diarrhea cases.[70–73] Studies using PCR assays on stool samples from AIDS patients with diarrhea have identified *Cryptosporidium* DNA in 18% to 77% of cases, significantly higher than with staining alone.[74–80] Thus, the prevalence is likely higher than suggested by earlier stool studies.

In a systematic review of cryptosporidiosis in low- and middle-income countries, overcrowding, diarrhea in the household, and animal contact were the major risk factors for infection.[81] Breastfeeding was protective. Surprisingly, there was no increased risk associated with water source.

A series of human challenge experiments was performed to determine the infectious dose of *Cryptosporidium* spp. The initial studies were performed in seronegative volunteers with different strains of *C. parvum* parasites (maintained in calves). The studies discovered a low infectious dose but considerable variability among isolates, with the dose infecting half of subjects ranging from approximately 1000 oocysts (*C. parvum* UCP strain), to approximately 100 oocysts (*C. parvum* Iowa strain), to approximately 10 oocysts (*C. parvum* TAMU strain, *C. hominis* TU502 strain).[82–84] Based on these data, even a single oocyst should result in infection in a portion of those exposed.[85] When the volunteers were rechallenged, they had a higher infectious dose.[86] They also developed less severe manifestations in that they were less likely to shed organisms but frequently developed symptomatic illness, with oocyst shedding detectable only with flow cytometry. When volunteers who were seropositive were challenged, the infectious dose was 20- to 50-fold higher.[87] Because the infectious dose is low, transmission can readily occur from exposure to low doses, such as might be found in waterborne outbreaks or in person-to-person spread.

Oocysts of *Cryptosporidium* are relatively resistant to environmental conditions. Oocysts can remain infectious for at least 6 months if kept moist, but viability decreases rapidly with desiccation.[48,88,89] Oocyst viability does not decrease with storage at temperatures between 0°C and 20°C. Viability decreases over a few hours with freezing (−20°C or lower). The oocysts can also be killed by heat, including pasteurization and microwave heating.[90] Oocysts are highly resistant to chlorination. For example, 80 ppm chlorine inactivated only 90% of oocysts after 90 minutes of incubation,[91] and the concentrations in tap water (e.g., 5 ppm) had no effect.[92] The sensitivity of oocysts to chlorine is further decreased in the presence of fecal contamination.[93] Even incubation of oocysts for up to 2 hours in household bleach failed to decrease infectivity.[94] By contrast, oocysts are sensitive to hydrogen peroxide, ozone, and ultraviolet radiation. Sunlight decreased oocyst viability up to 90%, but the effects vary with water turbidity, radiation intensity, the presence of biofilms, and time.[95–98]

Surveys have demonstrated that many sources of drinking water were contaminated with oocysts before treatment.[43,99–104] Oocysts are more frequent and at higher densities in water contaminated with agricultural runoff, sewage, urban runoff, or recreational use. However, up to 39% of apparently pristine sources were contaminated. Organisms found in the water included both *C. hominis* and *C. parvum* genotypes, in addition to animal species.[102,103] Even groundwater can be contaminated. Low-grade contamination has also been documented in samples of treated water, but this has been decreasing with improvements in water standards.[105]

Numerous outbreaks of cryptosporidiosis have been linked to contaminated drinking water.[43,44,104,106,107] The largest documented waterborne outbreak of diarrhea occurred in Milwaukee in 1993.[9] One of two city water treatment plants was contaminated. More than 600 cases of *Cryptosporidium* infection were confirmed by parasitologic examination. Based on telephone surveys, diarrhea episodes were more widespread, with an estimated 403,000 people developing a diarrheal illness. Of interest, water quality never failed to meet the standards current at the time for turbidity and fecal coliform counts. Many of the waterborne outbreaks, including the outbreak in Milwaukee, have been caused by *C. hominis*.[44,107,108] Thus, the outbreaks are thought to result from fecal contamination of drinking water. Other outbreaks are associated with *C. parvum*.[104] Most outbreaks of *C. parvum* can be tied to contamination of the watershed with agricultural runoff.[44,104] Over the past decades, there has been a marked reduction in the number of outbreaks and cases associated with drinking water in the United States, but *Cryptosporidium* remains one of the more common causes of waterborne outbreaks of disease.[109] Similarly, there has been a marked decrease in cryptosporidiosis in England and Wales associated with improved water standards.[110]

Outbreaks of cryptosporidiosis associated with contaminated recreational water are common.[43,45,106] The number of outbreaks increased throughout the 1990s.[53,54,111] In 2007 approximately 5700 people in Utah developed cryptosporidiosis associated with contamination of 450 swimming pools with an epidemic strain of *C. hominis*.[112] *Cryptosporidium* is now the most common organism associated with waterborne-disease outbreaks associated with recreational water in the United States.[54] In fact, small outbreaks appear to be an important source of endemic cryptosporidiosis.[45] Swimming is an important risk factor for cryptosporidiosis, and public swimming pools are frequently contaminated with *Cryptosporidium*.[43,44,113–116] Outbreaks have also involved lakes, rivers, beaches, and fountains.[43,117,118] The concentration of chlorine in pool water and limited filtration are often insufficient to disinfect the water. Not surprisingly, most outbreaks associated with fecal accidents are attributable to *C. hominis*.[54]

Foodborne infection occurs less frequently.[119] Well-documented outbreaks have been tied to contaminated apple cider, unpasteurized milk (cow and goat), salads, raw produce, and shellfish.[119–123] In resource-poor countries, oocysts are commonly found on vegetables.[124] Oocysts have been frequently identified in shellfish and in flies, but their role in transmission to humans is not clear.[125,126]

Oocysts of *Cryptosporidium* are immediately infectious when shed. Thus, *Cryptosporidium* is associated with direct person-to-person spread. Person-to-person transmission was initially recognized in outbreaks associated with contact with daycare centers.[127,128] There are also documented cases of nosocomial transmission.[129–132] The risk of transmission from adult patients is small with standard precautions.[133] However, contact with a person ill with diarrhea or with children in diapers remains a major risk factor for cryptosporidiosis.[44,117]

Secondary transmission within households is also common.[44,134,135] For example, in a study of household contacts of children with cryptosporidiosis in Brazil, Newman and coworkers noted secondary cases in 18 of 31 households (58%) and involving 19% of household members.[134] In daycare-associated outbreaks, secondary transmission is common.[127,128] By contrast, few cases in adults or school-aged children were associated with secondary transmission.[135,136]

Cryptosporidiosis in high-income countries is also associated with travel to resource-poor countries.[44,117,137,138] This was first recognized in Finnish travelers to Russia and was thought to reflect contamination of drinking water.[139] *Cryptosporidium* was thought to rarely cause traveler's diarrhea. However, in studies using molecular detection methods, the actual rates were up to 6% of cases.[140–142] Most travel-associated cases are caused by *C. hominis*.[44,138] However, a number of cases of *C. viatorum* have been reported in travelers returning to England from India.[143]

Sexual transmission has been postulated to occur. Among human immunodeficiency virus (HIV) patients, men who have sex with men are more likely to develop cryptosporidiosis.[144–146] Transmission is associated with anal-genital sex and the number of sex partners.[144] *Cryptosporidium* transmission among men who have sex with men shares risk factors and may occur concurrently with other fecal-oral pathogens, such as *Shigella*.[147]

C. parvum was thought to infect primarily domestic animals, with zoonotic transmission to humans. However, molecular studies have demonstrated that many of the human *C. parvum* infections are caused by subtype IIc, which typically only infects humans.[18,26,148] Animal contact is also associated with acquisition of *C parvum* in sporadic cryptosporidiosis and occasional outbreaks.[33,44,117,149–151] Cryptosporidiosis is common among veterinary students.[152–154] In addition to cattle, sheep, goats, pigs, and pets have also been implicated in zoonotic infection.[150,155,156]

The host immune response plays a key role in susceptibility. In studies from resource-poor countries where there is heavy exposure, most cases of cryptosporidiosis develop in young children, presumably because of rapid development of immunity.[134,157] Human challenge studies document resistance to infection associated with previous challenge or prechallenge immunity, as documented by anti-*Cryptosporidium* antibodies.[86,87] There is also strong evidence of an increased frequency of infection in patients with altered cellular immunity.[158] Among AIDS patients with diarrhea, *Cryptosporidium* was found in up to three-fourths of patients with chronic diarrhea from resource-poor countries.[77,159] In a waterborne outbreak affecting a drug treatment center, only 190 of 1392 (13.6%) HIV-negative patients developed cryptosporidiosis, compared with 104 of 339 (30.7%) infected with HIV.[160] Among those with HIV, the infection rate varied with the CD4 cell count, ranging from 23% of those with CD4 cell counts greater than 1000 cells/μL to 46% of those with CD4 cell counts less than 100 cells/μL. However, infection was not more frequent in HIV patients during the Milwaukee outbreak.[161] Cryptosporidiosis has also been noted in other immunodeficient hosts, including patients with primary immunodeficiencies, organ transplants, cancer, and diabetes.

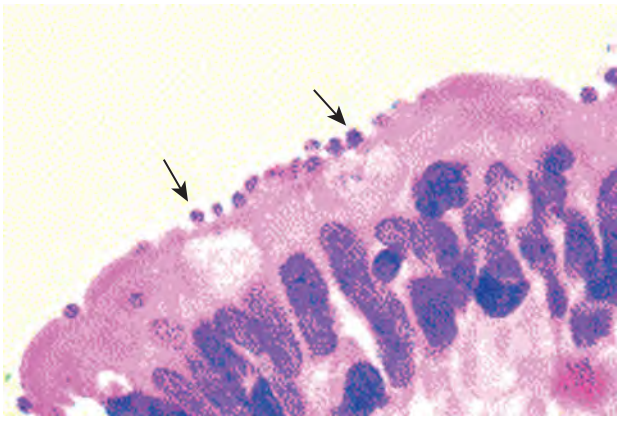

FIG. 282.2 Intestinal biopsy specimen showing *Cryptosporidium* intracellular forms (e.g., trophozoites and merozoites) (arrows) inside the surface of the intestinal epithelial cells. *(From Petri WA. Therapy of intestinal protozoa.* Trends Parasitol. *2003;19:523–526.)*

PATHOLOGY AND PATHOGENESIS

Cryptosporidium organisms are found within parasitophorous vacuoles in the microvillus layer of the epithelial cells (Fig. 282.2). In immunocompetent individuals, the organisms are localized primarily to the distal small intestines (e.g., terminal ileum) and proximal colon. However, studies from Uganda documented frequent infection of the respiratory tract in apparently immunocompetent children and adults.[162–164] In immunodeficient hosts, the parasites have been identified throughout the gut, in the biliary tract, and even in the respiratory tract.[165,166] Children with persistent cryptosporidiosis may have villous atrophy and a mild increase in lamina propria lymphocytes.[167] The distribution of parasites is limited and often spares the proximal small intestines. Heavier infection is associated with villous atrophy, crypt hyperplasia, and marked infiltration with lymphocytes, plasma cells, and even neutrophils,[168–172] and is also associated with extraintestinal involvement.

Villous atrophy and crypt hyperplasia are thought to reflect epithelial cell turnover.[171,173,174] Although infection of epithelial cells stimulates antiapoptotic mechanisms in infected cells, there is increased apoptosis in adjacent cells that is likely mediated by the interaction of Fas and Fas ligand.[175–178] Increased epithelial cell apoptosis has been demonstrated in biopsy specimens from infected intestines.[169] In vitro infection models show that infection initially stimulates antiapoptotic signals, but after 24 hours of infection, proapoptotic molecules dominate.[179,180] Furthermore, as the organisms complete their cycle, they cause necrotic death of the infected cells.[181,182] The resultant loss of villous surface was, in turn, associated with decreased expression of glucose-stimulated sodium pumps.[174] Similarly, loss of villous surface area has been demonstrated in human infection as D-xylose malabsorption.[171,183] Studies of AIDS patients with severe cryptosporidiosis have also demonstrated malabsorption of bile acids, vitamin B_{12}, and fatty acids.[171,184,185] Metabolic studies of AIDS patients with chronic cryptosporidiosis demonstrate fat wasting with a decreased metabolic rate, consistent with decreased absorption.[186]

Cryptosporidiosis is characterized clinically by watery diarrhea and malabsorption. The physiologic processes that are thought to account for these symptoms include sodium malabsorption, electrogenic chloride secretion, and increased intestinal permeability. Increased permeability may result in decreased absorption of fluids and electrolytes and solute fluxes into the gut. Studies of AIDS patients with cryptosporidiosis have

demonstrated a direct correlation between the severity of disease and altered intestinal permeability, as measured by ratios of excretion in the urine of lactulose and mannitol.[171,183,187] Similar defects have been noted in children with cryptosporidiosis.[188,189] *Cryptosporidium*-induced defects in intestinal epithelial cell barrier function can be reversed by antiinflammatory cytokines, such as transforming growth factor-β.[190]

The voluminous, watery diarrhea resembles that of toxin-mediated illnesses, but no secretory activity was detected in formal studies. Prostaglandins, induced by tumor necrosis factor-α (TNF-α), mediate decreased sodium absorption and cause diarrhea in porcine and bovine cryptosporidiosis.[191–193] However, studies in volunteers and AIDS patients with chronic cryptosporidiosis did not demonstrate any correlation between expression or level of proinflammatory cytokines and symptoms.[194–197] Furthermore, prostaglandin inhibitors have not proven to be effective symptomatic therapy in human cryptosporidiosis.

Robinson and colleagues demonstrated a correlation between expression of the neuropeptide substance P and the presence and severity of diarrhea in volunteers challenged with *C. parvum* and AIDS patients with chronic cryptosporidiosis.[198] In monkey models, *Cryptosporidium* infection is associated with increased expression of substance P and its receptor, and substance P inhibitors blocked *Cryptosporidium*-induced intestinal permeability, glucose malabsorption, and chloride secretion.[199,200] Similarly, mice were protected against *C. parvum*–induced intestinal inflammation and illness by a substance P receptor antagonist.[201,202]

Infection of intestinal epithelial cells leads to activation of nuclear factor kappa B (NF-κB).[175,176] Upstream signals may include Toll-like receptor 2 (TLR2) and TLR4 signaling via MyD88 (myeloid differentiation primary response 88).[203–205] NF-κB then activates several hundred target genes, including genes for antiapoptotic molecules such as osteoprotegerin.[34,206,207] These antiapoptotic molecules allow the parasite to form and release merozoites before cell death.[176,179] However, NF-κB activation also leads to upregulation of a proinflammatory cascade, including expression of chemokines, chemokine receptors, and cytokines. Many of these effects are mediated by upregulation or downregulation of micro-RNAs, including let-7, miR-27b, and miR-98.[204,208–210] These mediate secretion of exosomes, containing defensins (LL-37 and β-defensin 2), which may limit infection. The parasite also secretes noncoding RNAs into the host cytoplasm, which are transported to the nucleus by host HSP70. This in turn leads to activation of host histone methylation, increased expression of proinflammatory molecules (e.g., interleukin [IL]-8, IL-6, and CXCL2), and decreased expression of other host molecules, including the alarmin IL-33.[211,212] Both biopsy specimens and stool studies from human infection also demonstrate increased expression of proinflammatory cytokines and markers of inflammation, including TNF-α, IL-1β, IL-8, and lactoferrin.[194,213–216] Chemokines, including IL-8 and CXCL10, are produced by the infected epithelial cells.[216–219] Among the chemokines, CCL20 actually is microbicidal to extracellular forms of *Cryptosporidium*.[220] It is downregulated by the parasite via upregulation of MiR21. Studies of infected human tissues noted that *Cryptosporidium* infection leads to upregulation of the TNF receptor family decoy receptor osteoprotegerin.[207] It was also detected in intestinal tissues after experimental infection. Its ligand, TRAIL (TNF-related apoptosis-inducing ligand), was able to eliminate infected cells in vitro, but this effect was reversed by osteoprotegerin, suggesting that TRAIL may be a key mediator of parasite clearance.[207]

HOST RESPONSE AND IMMUNITY

Both innate and adaptive immune responses are critical for control of cryptosporidiosis.[10,216,221–223] $CD4^+$ T cells play a key role in the adaptive immune response to cryptosporidiosis. In patients with HIV infection, cryptosporidiosis is usually self-limited in individuals with CD4 cell counts higher than 150/μL, is often chronic in patients with CD4 cell depletion to less than 100/μL, and can be fulminant in some of those with counts less than 50/μL.[80,160,224–226] Similarly, infection is chronic in mice without functional CD4 cells (e.g., nude mice, SCID mice, or RAG knockout mice).[221,223] These defects can be reversed by infusion of $CD4^+$ cells, particularly $CD4^+$ intraepithelial lymphocytes. Kirkpatrick and colleagues[227] noted an association between DQ alleles, which present antigen to $CD4^+$ T cells, and susceptibility to infection. Furthermore, resolution of cryptosporidiosis among AIDS patients in response to effective antiretroviral therapy (ART) is associated with an influx of CD4 cells into the intestines.[228] The role of other cell populations has been less clear. Although major histocompatibility complex (MHC) class I deficiency and CD8 depletion have little effect on murine cryptosporidiosis, there are associations of MHC class I types and human infection.[227] CD8 cells produce interferon-γ (IFN-γ) in response to *Cryptosporidium* antigens, and sensitized human CD8 T cells can clear parasites from infected cells in vitro.[229,230]

The role of antibody in the immune response to cryptosporidiosis is controversial.[231] Early studies noted cases of chronic cryptosporidiosis in patients with low antibody levels, but these studies did not exclude coexisting T-cell dysfunction. In animal models, inactivation of B cells by inactivation of the *muMT* gene did not affect clearance of cryptosporidiosis.[232] Treatment with high concentrations of anti-*Cryptosporidium* antibody did facilitate clearance in mice.[233] Anecdotes suggested that hyperimmune bovine colostrums might improve cryptosporidiosis in AIDS, but a large randomized, controlled trial demonstrated no clinical benefit and decreases in oocyst shedding only at very high doses.[234] High levels of serum and fecal antibodies to *C. parvum* have been found in AIDS patients with chronic cryptosporidiosis.[235,236] Studies of the fecal antibody response in volunteers challenged with *C. parvum* demonstrated specific fecal antibody in most volunteers.[237] However, the presence and timing of antibody correlated with oocyst shedding rather than clearance or resistance to infection. Similarly, cytokines such as TGF-β that stimulate immunoglobulin A (IgA) production often develop only after resolution of illness.[238] Studies have noted higher antibody responses to specific antigens in patients with acute versus persistent diarrhea, but this may have been a marker for prior exposure.[80,239,240] Also, specific antibody in breast milk was associated with decreased risk of infection in infants.[241] Thus the importance of antibody in cryptosporidiosis remains unclear.

Production of IFN-γ is a key mediator of the adaptive immune response to *Cryptosporidium*. In murine models, IFN-γ knockout mice develop chronic infection.[242,243] Furthermore, inactivation or depletion of IFN-γ causes further exacerbation of infection even beyond that noted with CD4 depletion.[244] Lymphocytes from persons who have recovered from cryptosporidiosis produced IFN-γ after antigen stimulation in vitro, including HIV patients.[230,245,246] Approximately half of volunteers challenged with *C. parvum* express IFN-γ in the intestinal mucosa.[247] Treatment with IFN-γ can directly activate intestinal epithelial cell lines to prevent *C. parvum* infection, but this effect was not confirmed with primary cells.[248] Similarly, inactivation of IL-12, the major factor stimulating production of IFN-γ, causes chronic infection.[249]

Surprisingly, IFN-γ expression in normal volunteers was limited to the subset with evidence of previous exposure (either seropositive before challenge or demonstrating resistance to infection).[247] Similarly, IFN-γ production by cells from HIV patients during active cryptosporidiosis and from Haitian children with active cryptosporidiosis was very low despite the fact that they had self-limited disease.[214,246] Thus, other factors appear to be involved in limiting human infection after initial exposure. In murine models, inactivation of IFN-γ expression resulted in only a mild chronic infection in BALB/c mice but fatal infection in C57BL/6 mice. Mild disease in BALB/c mice was associated with expression of IL-12, IL-4, and TNF-α.[243,250] In the absence of IFN-γ, IL-12 treatment only worsened cryptosporidiosis.[251] Similarly, IL-12 treatment of AIDS patients with chronic cryptosporidiosis was associated with gastrointestinal side effects.[252] IL-4 synergizes with IFN-γ in preventing infection of epithelial cells, and IL-4 knockout mice displayed prolonged oocyst shedding.[251,253,254] IL-4 treatment did not modulate infection in IFN-γ knockout mice and IL-4 expression did not correlate with protection in human volunteers.[251,255] By contrast, TNF-α limited infection in IFN-γ knockout mice, activated human epithelial cells to limit infection, but did not correlate with resolution in human infection.[194,248,256]

Studies have increasingly focused on the innate immune response to cryptosporidiosis.[223] Mononuclear phagocytes likely play a key role in control of cryptosporidiosis. Takeuchi and colleagues demonstrated that mice can be rescued from a fatal infection by type I macrophages.[257] Macrophages can be a source of IL-15 and IL-18, which have been implicated in control of infection. IL-18 stimulates IFN-γ production, natural killer (NK) cell activation, and secretion of defensins.[258–260]

Depletion of dendritic cells ($CD11c^+$) increased susceptibility to *C. parvum* infection in a mouse model, and dendritic cells stimulated with sporozoites and cocultured with $CD4^+$ and $CD8^+$ lymphocytes produce higher levels of IFN-γ, and the subsequent control of infection is sustained.[261] Dendritic cells were also important in a neonatal mouse system in which they limited infection in part via stimulation of Th1 responses.[261–263] NK cells have a role in parasite clearance in some animal models, and human NK cells can clear infection of cell lines in vitro.[223,264–266] In seronegative normal volunteers experimentally infected and AIDS patients recovering from cryptosporidiosis in response to ART, control of infection was associated with expression of IL-15.[197,255] This effect is likely mediated by activation of NK cells.[264] Mannose-binding lectin levels have been shown to be low in AIDS patients and children with cryptosporidiosis, and mice with the gene inactivated are more susceptible to infection.[267–271] Antimicrobial peptides seem to be a key host defense mechanism against the luminal forms of the parasite. β-Defensins and CCL20 are induced in response to *Cryptosporidium* infection and can kill the parasites in vitro.[220,259,260,272–274] More frequent or severe cryptosporidiosis has been noted in a number of primary immunodeficiencies.[158,275,276] Some cases have primary T-cell defects. However, recent descriptions primarily involve innate responses. For example, hyper-IgM syndrome, caused by a defect in CD40 ligand (also termed CD154), is associated with increased frequency and severity of *Cryptosporidium* infection.[276–279] This syndrome is associated with profound defects in the ability of antigen-presenting cells to produce IL-12 and TNF-α and to stimulate production of IFN-γ.[280] In murine models, recovery required expression of CD40 on donor spleen cells but not recipient epithelial cells.[281] Similarly, treatment of patients with hyper-IgM syndrome with CD40 agonist antibody can lead to resolution of severe forms of cryptosporidiosis in patients with CD40L defects.[282] The prevalence of cryptosporidiosis ranges from 6% to over half of cases. Furthermore, patients often developed biliary disease, primarily sclerosing cholangitis, which is usually due to *Cryptosporidium.* Studies using PCR assays have demonstrated that most biliary tract infections were caused by *Cryptosporidium.*[275] An association with sclerosing cholangitis and cryptosporidial infection has also been reported with other primary immunodeficiencies affecting T cells, including dedicator of cytokinesis 8 (DOCK 8) deficiency, and NIK (nuclear factor kappa B–inducing kinase) loss-of-function mutations.[283,284]

CLINICAL MANIFESTATIONS

Symptoms of cryptosporidiosis develop after a prepatent period, during which the parasites invade the intestinal epithelium and proliferate. Studies of immunocompetent individuals with discrete exposures (e.g., travelers, point-source outbreaks, or experimental infection) demonstrate a prepatent period of approximately 1 week.[9,83,84] There is, however, considerable variability, with a range of 1 to 30 days. This variability reflects, in part, strain differences among the organisms rather than dose.[83,285] Cryptosporidiosis is noted in both males and females. The age distribution varies considerably with the epidemiology of exposure. In low- and middle-income countries, most cases occur among children younger than 2 years.[10,11] This is thought to reflect both high rates of fecal-oral exposure in children and the development of immunity in older children and adults. In higher income countries, the prevalence is higher in children younger than 5 years, but the condition occurs in all age groups.[53] Waterborne epidemics in high-income countries affect people of all ages.[9,43,107,286] Because *Cryptosporidium* infects primarily intestinal epithelial cells, it is not surprising that diarrhea is the most common clinical presentation.[287] However, there are significant differences in the clinical presentation, depending on the host and parasite population. The major groups include immunocompetent individuals in high-income countries, children in low- and middle-income countries, and immunocompromised hosts (e.g., patients with AIDS or after organ transplantation).

Immunocompetent Individuals in High-Income Countries

Most case series of immunocompetent individuals from high-income countries have been associated with waterborne outbreaks, infection in travelers, animal contact, or infections of children in daycare and their contacts.[114,117,138,288,289] Immunocompetent adults most commonly present with diarrhea.[9,107,287] The diarrhea is usually described as watery but may also be described as mucoid. The median duration of illness in most case series is approximately 5 to 14 days but may range from 1 to 100 days. Data from Sweden noted a duration of less than 4 days from a survey after a waterborne outbreak, but more than 10 days for cases identified from laboratory records.[107,138] Accompanying symptoms are similar to those noted with other diarrheal illnesses, including abdominal cramps, nausea, vomiting, and fever. The frequency of abdominal pain varies among reports. In some studies, cryptosporidiosis was more frequently associated with respiratory symptoms.[163] Nearly half of cases develop recurrent symptoms after initial resolution.[107,135,290,291] Relapses may follow a diarrhea-free period of several days to weeks. The presence of chronic gastrointestinal symptoms similar to irritable bowel syndrome is not uncommon, including diarrhea, abdominal pain, and weight loss.[291–294] Other chronic sequelae include arthralgias, fatigue, and eye pain. Clinical illness can be severe among the elderly.[114,295,296]

Milder or even asymptomatic infection may also be very common. For example, seroconversion is more common than is clinically diagnosed disease.[297] Similarly, in waterborne outbreaks, only a minority of affected individuals presented for clinical care.[107,298] Patients identified by active case-finding or children with negative stool studies were less severely ill and had a shorter duration of diarrhea than laboratory-confirmed cases.

Childhood Diarrhea in Low- and Middle-Income Countries

Childhood diarrhea is the most common clinical manifestation of cryptosporidiosis in resource-poor countries. Investigators in Asia, Africa, and Latin America have carefully studied cryptosporidial diarrheal illness among children.[a]

These studies have demonstrated that cryptosporidiosis is common, with most children infected by age 2 or 3.[66,67] In a prospective, multicenter birth cohort study from Asia, sub-Saharan Africa, and Latin America, 65% of infants were infected by age 2.[64] In birth cohort studies that used PCR to detect organisms, most infections were subclinical.[66,67] The main clinical presentation is with an acute diarrheal syndrome similar to that seen with other enteric pathogens—with watery diarrhea, cramps, nausea, and vomiting. Signs of dehydration are common. Less common features may include abdominal pain, fever, shortness of breath, cough, and foul stools. Although most cases resolve within a few days, many affected individuals go on to develop prolonged, recurrent, or persistent diarrhea.[50,71,299,301–304] Thus, *Cryptosporidium* is among the more common causes of persistent diarrhea in resource-poor countries, causing approximately one-third of cases. It can also cause chronic diarrhea and malabsorption.[305,306] Furthermore, an episode of prolonged or persistent diarrhea, especially if caused by *Cryptosporidium,* is a marker for the onset of increased risk of recurrent episodes of diarrhea, weight loss, and premature death.[11,301,303,304,307] A long-term follow-up study of children with onset of cryptosporidiosis before age 1 year suggested an association with poorer physical fitness and poorer cognitive development that persists for years.[308,309] Molecular studies have demonstrated clinical differences between *Cryptosporidium* species and subtypes. In some studies, *C. hominis* is associated with more severe disease (more dehydration, longer duration, more oocysts shed) and *C. meleagridis* with milder disease.[26,32,310,311]

Cryptosporidium and Malnutrition

Studies of childhood diarrhea in resource-poor countries have demonstrated an association between cryptosporidiosis and malnutrition. Cryptosporidiosis is more severe in children with malnutrition.[10] For example, most deaths occur in malnourished children.[71,312,313] Early studies did not clearly distinguish the effects of cryptosporidiosis on nutritional status from the effects of malnutrition on cryptosporidiosis. Studies prospectively examining both nutritional status and *Cryptosporidium* infection in cohorts of children demonstrated significant differences in nutritional status before *Cryptosporidium* infection.[64,301] Onset of cryptosporidiosis was associated with growth faltering, with a decrease of 300 to 400 g.[64,67,301,314,315] Older children or children in sites with better

[a]References 26, 51, **61**, **64**, **66**, **67**, 299, 300.

nutrition eventually recovered and experienced catch-up growth. In contrast, children infected before 1 year of age or those in South Asia often never recovered. Furthermore, even subclinical infection (i.e., no diarrhea) was associated with growth faltering. Animal models have confirmed the bidirectional interaction between malnutrition and cryptosporidiosis.[316] The burden of disease from the nutritional impact of cryptosporidiosis is thought to be greater than that of diarrhea.[65] Thus, *Cryptosporidium* infection is a cause of acute malnutrition, and the long-term consequences of this interaction are likely to be worse in those infected in infancy or with previous malnutrition.

Cryptosporidiosis in HIV Infection

HIV infection has been the most common host defense defect associated with cryptosporidiosis. Before the advent of effective antiretroviral combinations, most patients diagnosed with cryptosporidiosis had underlying HIV infection.[145] However, the incidence of cryptosporidiosis in HIV has dramatically decreased with improvements in combination antiretroviral therapy (cART).[317,318] However, *Cryptosporidium* remains the most prevalent enteric pathogen among those with poor access or adherence to ART.[76–78,80,319,320] For example, in Kenya, more than a third of HIV patients with or without diarrhea shed *Cryptosporidium* oocysts.[80] The clinical manifestations of cryptosporidiosis in HIV patients are variable. Among patients with CD4 cell counts greater than 150 cells/μL, most cases of cryptosporidiosis are self-limited, similar to those in normal hosts.[169,321–324] However, even these cases are more likely to relapse if the cellular immune response deteriorates. Surprisingly, in population-based studies, a substantial portion of *Cryptosporidium* infections in HIV patients are asymptomatic, and some cases are mild and self-limited even in patients with advanced HIV infection.[320,325,326] Other patients develop a chronic diarrheal illness. The chronic diarrhea is associated with frequent, foul-smelling, bulky stools. Most patients experience weight loss. Not surprisingly, studies have demonstrated nutrient malabsorption. Voluminous watery diarrhea or cholera-like illness develops in a minority of patients. The clinical picture is often confused by other concomitant opportunistic infections, including microsporidiosis, disseminated *Mycobacterium* infection, or cytomegaloviral colitis.[145,169] There may be slight differences in the clinical manifestations depending on the parasite species, with milder disease caused by *C. meleagridis* and some *C. hominis* subtypes.[325]

Cryptosporidiosis in AIDS is also associated with extraintestinal disease, including involvement of the biliary and respiratory tract.[145,327–331] Respiratory tract involvement is often asymptomatic but may also manifest as pulmonary infiltrates with dyspnea.[163,164,327,328] Biliary tract involvement in cryptosporidiosis has been limited to patients with profound immunodeficiency. Biliary involvement correlated with a low CD4 cell count and a markedly shortened survival.[145,329] Patients may present with acalculous cholecystitis, sclerosing cholangitis, papillary stenosis, or pancreatitis.[145,329,330,332] Most patients present with right upper quadrant abdominal pain, which may be intermittent and colicky. Laboratory studies characteristically reveal elevated levels of alkaline phosphatase. Levels of bilirubin and transaminases are often elevated. In patients with associated pancreatitis, amylase and lipase are increased. Ultrasound examination may reveal dilatation of the biliary duct and/or signs of gallbladder inflammation. Magnetic resonance cholangiopancreatography can usually confirm the diagnosis.[331] However, in many patients an endoscopic retrograde cholangiopancreatographic evaluation is required in order to make the anatomic diagnosis. Biopsy specimens of the biliary ducts, staining of the bile, or stool studies may demonstrate the parasites. Many cases of biliary disease will reveal evidence of coinfection with cytomegalovirus or microsporidia.

Transplant Patients

Cryptosporidiosis is being increasingly recognized in organ transplant patients.[333–337] Cases typically manifest a few years after initial transplantation with persistent diarrhea, usually lasting about 10 days before diagnosis. Cryptosporidiosis appears to be more frequent in patients on tacrolimus than on cyclosporine, and the former typically have elevated tacrolimus levels.[333,335] Renal transplant recipients often demonstrate evidence of acute kidney injury, thought to be due to a combination of volume depletion and tacrolimus toxicity. Cryptosporidiosis is particularly problematic among transplant patients in areas highly endemic for cryptosporidiosis.[335,338] For example, a study in Pakistan demonstrated *Cryptosporidium* oocysts with acid-fast staining in 343 of 644 (53%) renal transplant patients with diarrhea.[338] Extraintestinal disease, including biliary and pulmonary disease, is noted in about 10% of transplant patients with cryptosporidiosis.[337]

DIAGNOSIS

Parasites were first demonstrated by means of histologic staining of intestinal tissues. The organisms are found along the surface of the epithelial cells and may appear to be in the lumen (see Fig. 282.2). The intracellular forms stain purple with hematoxylin. Tissues are available only after invasive procedures, and the organisms are not consistently identified in biopsy specimens. *Cryptosporidium* infection was traditionally diagnosed through microscopic examination of stool. In general, stools are preserved in 10% buffered formalin.[27,339,340] Fresh stools can also be tested but are infectious to laboratory personnel. Polyvinyl alcohol interferes with staining techniques and is not recommended. Frozen stools can be used for some immunoassays and nucleic acid tests. Potassium dichromate (2.5%) can also be used to preserve organisms without decreasing oocyst viability.

A number of concentration methods have been attempted. The formalin–ethyl acetate method is commonly used in clinical laboratories. However, oocysts may fail to sediment if centrifugation speed or time is not increased.[27,339,340] Immunomagnetic beads can be used to isolate and concentrate organisms and improve sensitivity of stool examination.[341]

On wet mounts oocysts are small, 4 to 6 μm in diameter, and similar in size and shape to yeast forms normally found in stool. They do not stain well with iodine or trichrome and cannot be differentiated from yeast forms with Giemsa staining. Thus, traditional approaches to stool examinations usually cause the organism to be missed. Many laboratories still do not routinely test all stools for *Cryptosporidium*.[27,57,58]

Differential staining was first noted with acid-fast stains. Oocysts stain pink or red, whereas yeast cells and fecal debris stain green or blue (Fig. 282.3). The most commonly used stain is a modification of the Ziehl-Neelsen stain.[27,339,340] The sensitivity of stool examination with acid-fast staining remains poor, with fewer cases detected than with antigen-detection, fluorescent, or molecular methods.[27,340,342,343] Fluorescent stains (e.g., auramine-rhodamine) can be read more quickly than other acid-fast stains and may have improved sensitivity (see Fig. 282.3). However, these assays are plagued by false-positive results. All of the acid-fast stains detect other parasites that may cause similar illnesses (e.g., *Cystoisospora* and *Cyclospora*).

Immunofluorescence assays (IFAs) using oocyst-specific monoclonal antibodies can be used to test for cryptosporidiosis.[27,339,340,342] IFA is more sensitive than acid-fast staining. Direct immunofluorescence using monoclonal antibodies is now a gold standard for stool examination. Some of the commercial IFAs (Merifluor *Cryptosporidium/Giardia,* Meridian Bioscience, Cincinnati, OH) also include antibodies to *Giardia* in addition to *Cryptosporidium.*

Antigen detection assays are being increasingly used for stool diagnosis.[58,339,340,344] Commercial kits for *Cryptosporidium* are available in enzyme-linked immunosorbent assay (ELISA) and immunochromatographic formats.[340,341,345] The ELISA kits for *Cryptosporidium* have generally performed well for diagnosis of cryptosporidiosis, with sensitivities ranging from 66% to 100% and excellent specificity.[27,340,341,345] Most studies suggest improved sensitivity compared with microscopic methods, and these assays are being increasingly used by clinical laboratories.[57,58,339] Quality control, however, may be an issue. For example, antigen detection kits have been associated with pseudo-outbreaks stemming from false-positive results, and the results may be less reliable in community hospitals.[345–347] Some commercial ELISA kits also test for *Giardia* and *Entamoeba* antigens. The immunochromatographic tests are rapid tests for *Cryptosporidium* and *Giardia* antigen. Antigen assays as a group have the advantage of not requiring skills in microscopic identification of organisms.

Molecular methods for *C. parvum* DNA are increasingly used in diagnostic laboratories.[339,344] They also have increased sensitivity compared with microscopic studies of stool, and nearly double the number of cases diagnosed compared with stool assays.[348] Several multiplex

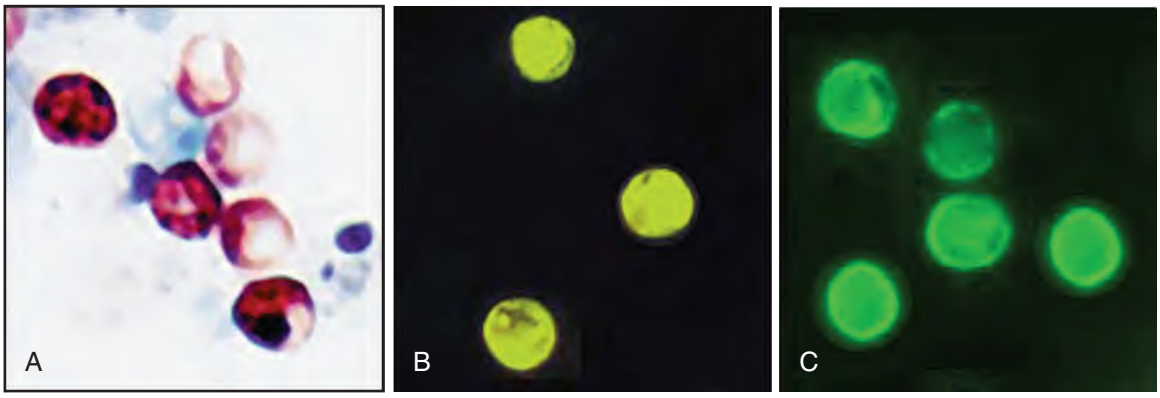

FIG. 282.3 Cryptosporidiosis is diagnosed through demonstration of organisms in stool samples. This can be done with immunoassays for antigen, with microscopic demonstration of the organisms, or through nucleic acid amplification. Because the organisms are similar in size and shape to yeast normally found in stool, differential stains are required to identify the organism. These may include modified acid-fast staining (A) showing that the oocysts are red organisms; fluorescent stains, such as auramine-rhodamine (B); or immunofluorescence (C) (green organisms). *(From the Centers for Disease Control and Prevention. DPDx. Cryptosporidiosis.* http://www.dpd.cdc.gov/dpdx/HTML/Cryptosporidiosis.htm.*)*

molecular assays have been approved by the US Food and Drug Administration (FDA), including Luminex xTAG gastrointestinal pathogen panel, BioFire FilmArray gastrointestinal panel, NanoCHIP gastrointestinal panel, and BD Max parasitic panel.[349–352] EasyScreen is available in Australia. All detect multiple parasites. The Luminex, NanoCHIP, and BioFire assays also detect a range of other bacterial and viral pathogens. In general, these assays are quite sensitive and specific.[339,344,353] However, they are costly and may generate positive results that are not clinically relevant.

MANAGEMENT

Supportive therapy is a key component in the management of cryptosporidiosis. As is the case for all causes of diarrhea, replacement of fluids and electrolytes is a critically important first step in management. Oral rehydration is preferred, but severely ill patients may require parenteral fluids. Fluids should include sodium, potassium, bicarbonate, and glucose. Cryptosporidiosis is characterized by preferential loss of mature epithelial cells at the tips of the villi, and enzymes expressed on these cells, including lactase, are lost. Thus, supportive care should include a lactose-free diet. In contrast to glucose-stimulated sodium pumps, which are expressed on the villus tips, glutamine-stimulated sodium absorption is not affected.[354] Glutamine supplementation, usually given in the form of alanyl-glutamine, may improve fluid absorption.[316,355,356] Nutrition remains important. As is the case for other forms of childhood diarrhea, breastfeeding should be continued and zinc supplementation provided for children in low-resource settings.[357,358] Although some patients have received parenteral nutrition, oral feeding is as effective as parenteral nutrition in those who can tolerate it.[359]

Cryptosporidiosis is associated with increased intestinal transit, which could interfere with absorption of fluids, electrolytes, and drugs.[183,360] Thus, antimotility agents play a key role in therapy. Opiates, such as loperamide and diphenoxylate/atropine combination, may ameliorate mild symptoms, but their efficacy is limited in severe disease. More potent opiates, including tincture of opium, may work in patients who have not responded. Several trials have examined octreotide therapy in AIDS patients with diarrhea. Overall, octreotide was effective but not consistently more effective than other oral antidiarrheal agents.[361,362] Because of its high cost, its use is generally limited to refractory cases. The enkephalinase inhibitor racecadotril (acetorphan) has been widely used as an antisecretory drug in watery diarrhea in Europe and many other countries but is not approved in the United States.[358] The results of a single study suggested that racecadotril is more effective than somatostatin in AIDS-associated diarrhea.[363] However, none of the studies of racecadotril specifically addressed cryptosporidiosis.

For immunodeficient patients, restoration of the immune response should be pursued. For AIDS patients with chronic cryptosporidiosis, effective ART can result in dramatic improvement in diarrhea.[197,364–368] This should take the form of recommended combinations of potent antiretroviral drugs. The HIV protease inhibitors have anticryptosporidial activity in vitro and reduced infection by up to 90% in an animal model.[369,370] However, they are no longer recommended as first-line HIV therapy. Despite improved immune response, one study documented continued early mortality in patients treated with just antiretrovirals.[368] Several studies have combined ART with antiparasitic agents.[197,371,372] Although unproven, this approach could in theory improve the response to both treatments.

Solid-organ and stem cell transplant patients also present with severe disease similar to that seen in patients with AIDS, including sclerosing cholangitis.[333–337] When possible, immunosuppression should be minimized. Cryptosporidiosis has been associated with elevated levels of tacrolimus, which may worsen the course of cryptosporidiosis.[333,335] Some patients have improved when switched from tacrolimus to cyclosporine. There are anecdotes of responses to antiparasitic drugs, including nitazoxanide, paromomycin, and/or azithromycin.[334,335,337] Most patients required a prolonged regimen and/or combinations of drugs.

Biliary involvement in cryptosporidiosis is a severe complication seen in immune-compromised hosts including patients with AIDS, transplant patients, and some patients with primary immunodeficiencies (e.g., hyper-IgM syndrome). Initial therapy should focus on improving immune function with cART in patients with AIDS, moderating immunosuppression in transplant patients, or agonist antibody to CD40 in patients with hyper-IgM syndrome.[282,330,337,373] Optimal treatment often also requires invasive approaches. Acalculous cholecystitis should be treated with cholecystectomy.[374] Patients with sclerosing cholangitis or papillary stenosis may require endoscopic retrograde cholangiopancreatography. Sphincterotomy often results in temporary improvement. However, symptoms often recur unless a stent is placed.[145,375]

Antiparasitic Drugs

The role of antiparasitic therapy in cryptosporidiosis has been difficult to demonstrate.[10,376] Initial attempts to screen drugs for anticryptosporidial activity in vitro and in animal models have met with limited success. For some drugs, resistance is attributable to target insensitivity. For example, the *Cryptosporidium* dihydrofolate reductase–thymidylate synthetase contains novel amino acids at sites associated with antifolate resistance in other species.[377] A second reason for drug resistance stems from the unique location of the parasite within the host cell, but segregated in the parasitophorous vacuole, which does not communicate with the epithelial cell cytoplasm.[41]

No antiparasitic drug has proved reliably curative in severely immunocompromised patients.[378] Because cryptosporidiosis is eventually self-limited in immunocompetent hosts and can be variable in immunocompromised hosts, controlled trials are critically important. In compromised hosts, however, most trials have not been designed to detect partially active agents. Few of the studies of AIDS patients have rigorously excluded common coinfection with *Mycobacteria,*

microsporidia, or cytomegalovirus, all of which may mask the effects of anticryptosporidial treatments.[379] Although a meta-analysis of seven randomized, controlled clinical trials found no clear evidence for efficacy of antiparasitic agents in the management of cryptosporidiosis in compromised hosts,[380] partially active drugs (which might prove useful in combination or in situations in which the patient's immune response can be boosted) may have been labeled as ineffective.

Nitazoxanide is a nitrothiazolyl-salicylamide, broad-spectrum antiparasitic drug.[381] Initial studies noted efficacy versus *Taenia saginata* and *Hymenolepis nana.* However, clinical development progressed only after antiprotozoal activity was demonstrated in the early 1990s. Nitazoxanide suspension was approved in the United States for treatment of cryptosporidiosis and giardiasis in children in 2002. Nitazoxanide inhibits growth in vitro at concentrations of less than 10 μg/mL.[382,383] The metabolite tizoxanide is less active, but tizoxanide glucuronide is nearly as active as the parent compound. Nitazoxanide decreased parasite numbers but was not curative in gnotobiotic pigs and was not effective in SCID mice depleted of IFN-γ.[382]

A randomized, controlled study of nitazoxanide in HIV patients with cryptosporidiosis was performed in Mexico; it compared doses of 500 mg twice daily, 1 g twice daily, or placebo for 2 weeks.[384] Among HIV patients with CD4 cell counts greater than 50 cells/μL, 10 of 14 (71%) responded to 1 g/day and 9 of 10 (90%) to 2 g/day, compared with 3 of 15 (20%) treated with placebo. By contrast, the response was no better than placebo in patients with CD4 cell counts of 50 cells/μL or lower.

Three randomized trials were performed in patients with cryptosporidiosis who were not infected with HIV. An outpatient study was performed in Egypt in adults and children with cryptosporidiosis and prolonged diarrhea (mean duration, 13 days).[385] Adults, children aged 4 to 11 years, or children aged 1 to 3 years received nitazoxanide in doses of 500 mg, 200 mg, or 100 mg twice daily or matching placebo for 3 days. Diarrhea resolved by day 7 in 39 of 49 (80%) in the nitazoxanide group, compared with 20 of 49 (41%) in the placebo group. Oocysts were no longer detected in 33 of 49 (67%) treated with nitazoxanide, compared with 11 of 50 (22%) treated with placebo. A second trial of outpatients in Egypt compared nitazoxanide tablets, suspension, and placebo. The response rate was 26 of 28 (93%) in the nitazoxanide arms, compared with 10 of 27 (37%) in the placebo arm.[386] Parallel randomized trials of HIV-infected and HIV-negative children hospitalized with chronic cryptosporidiosis were performed in Zambia.[305] Nearly all the participants had moderate-to-severe malnutrition and persistent diarrhea or chronic diarrhea. All children were treated with nitazoxanide suspension (100 mg twice daily for 3 days) or matching placebo. In the trial of HIV-negative children, diarrhea had resolved by day 7 in 14 of 25 (56%) of those treated with nitazoxanide, compared with 5 of 22 (23%) for those treated with placebo. Follow-up stool studies were free of oocysts in 13 of 25 (52%) children in the treatment group, compared with 3 of 22 (14%) in the placebo group. Most of those who did not respond became well after a second course, given on an open-label basis. Significantly more HIV-negative children died in the placebo group. A fourth trial in hospitalized children in Egypt noted an 87% cure rate with nitazoxanide compared with 69% with paromomycin.[387]

Among HIV-infected children, Amadi and colleagues noted no significant differences in clinical and parasitologic responses or in mortality rate with nitazoxanide treatment.[305] A subsequent placebo-controlled trial in children with AIDS and cryptosporidiosis did not show significant improvement with nitazoxanide compared with placebo, despite use of higher doses and continuation of treatment for 2 weeks.[388] A compassionate use program noted that AIDS patients could be treated safely with nitazoxanide at doses of 1 g twice daily for at least 2 weeks.[389]

Paromomycin is an orally administered nonabsorbable aminoglycoside originally approved in the 1960s as a luminal amebicide. Initial in vitro studies noted poor activity against *C. parvum* with inhibitory concentrations in the range of 100 to 500 μg/mL. However, when AIDS patients with cryptosporidiosis were treated with available antiparasitic drugs, some improved when treated with paromomycin. The first 12 published case series of AIDS patients treated with paromomycin included more than 300 patients, with a response rate of 67%.[145] In many of those with initial improvement, relapse occurred later. Two randomized, controlled trials have examined the effects of paromomycin in AIDS patients with cryptosporidiosis. In a small placebo-controlled trial incorporating quantitation of oocyst excretion, the paromomycin arm demonstrated a significant reduction in oocyst shedding (about 70%), decreased stool frequency in those treated with paromomycin, but no cures.[390] Biliary tract involvement and *Mycobacterium* coinfection were common in those not responding. Hewitt and colleagues[391] compared paromomycin with placebo in a trial including 35 AIDS patients. There was no difference between groups when analysis of those on treatment was performed, but dropouts occurred only in the placebo arm. By intent-to-treat analysis with dropouts grouped with failures, the response rate was similar to that in the previous trials, with a trend favoring paromomycin over placebo.[392] The trial was prematurely terminated because of poor enrollment and was not powered to detect limited response rates. Limited efforts were made to exclude coinfections. Dose escalation demonstrated no further improvement with higher doses, and higher doses have been associated with gastrointestinal toxicity and ototoxicity.[392]

Macrolide antibiotics, including spiramycin, azithromycin, roxithromycin, and clarithromycin, have some activity against *Cryptosporidium.*[376] Sáez-Llorens and colleagues[393] reported shorter duration of symptoms and oocyst shedding when children were treated with 100 mg/kg/day of spiramycin, but a second trial showed no effect.[394] In a randomized placebo-controlled trial among intravenous drug users, Huang and colleagues reported a higher cure rate with acetyl-spiramycin, especially when combined with garlicin.[395] However, no information is provided on HIV status despite this being a high-risk population. In an unpublished study, the AIDS Clinical Trials Group (ACTG) conducted a randomized, controlled trial in 75 AIDS patients, comparing spiramycin with placebo, but noted that spiramycin was not significantly better than placebo.[376] In a second unpublished trial, intravenous spiramycin was associated with significantly decreased oocyst shedding and a partial response in 75% of participants.[376] However, there were high rates of adverse events, including drug-associated intestinal injury.[396]

Azithromycin has some activity against *Cryptosporidium* in vitro and in animal studies. Uncontrolled reports have noted improvement in cryptosporidiosis among HIV and transplant patients treated with azithromycin.[334,337,397] In a placebo-controlled, multicenter trial, AIDS patients with cryptosporidiosis were randomly assigned to receive azithromycin, 900 mg orally daily, or placebo. Overall, oocyst shedding, stool frequency, and weight loss were not significantly different.[398] A subsequent pilot trial of intravenous azithromycin also did not demonstrate changes in stool frequency or oocyst shedding.[399] A pilot study of Egyptian schoolchildren suggested more rapid resolution with azithromycin treatment.[400]

Rifamycin antibiotics have some activity against *Cryptosporidium* in vitro.[376] Rifaximin, a nonabsorbable rifamycin, is approved by the FDA for the treatment of traveler's diarrhea. Although it has some activity against cryptosporidiosis in HIV patients, its efficacy has never been assessed in controlled studies.[401,402] Rifabutin is active against *Cryptosporidium* in vitro, and use of rifabutin for prophylaxis of *Mycobacterium avium* infection in AIDS patients was associated with a decreased incidence of cryptosporidiosis.[403–405]

Anecdotal reports noted improvement in chronic cryptosporidiosis in patients treated with oral anti-*Cryptosporidium* immunoglobulin preparations. However, three controlled trials have examined oral bovine anti-*Cryptosporidium* immunoglobulin preparations in cryptosporidiosis. Bovine anti-*Cryptosporidium* immunoglobulin did not significantly decrease symptoms or oocyst shedding in experimental infection of volunteers.[406] In a large trial of bovine anti-*Cryptosporidium* immunoglobulin for cryptosporidiosis in AIDS patients, there was no effect on symptoms, and oocyst shedding decreased only slightly at a dose of 20 g/day.[234] At higher doses, oocyst excretion decreased, but the immunoglobulin preparation caused diarrhea. A third trial was stopped with only five participants after demonstrating similar improvement in hyperimmune and control colostrum.[407]

Because individual drugs have limited activity, studies have investigated the effects of combinations. In vitro studies have suggested that azithromycin and rifabutin may enhance the parasiticidal activity of nitazoxanide.[404] Lee and colleagues[408] studied azithromycin and nitazoxanide in gnotobiotic pigs infected with *C. hominis.* They noted optimal parasitologic responses with just nitazoxanide, but combination

therapy with azithromycin improved the clinical response. There are a number of anecdotes of transplant and AIDS patients being treated with antiparasitic combinations.[337] Smith and colleagues[409] conducted a prospective pilot study of the combination of paromomycin combined with azithromycin in AIDS patients with chronic cryptosporidiosis. Overall, there was a 2-log decrease in oocyst shedding, but few patients were cured. Clinical failures were associated with biliary disease, coinfection with other enteric pathogens (especially cytomegalovirus), or side effects of the medications. Combination therapy therefore warrants further study.

In recent years, the increased recognition of cryptosporidiosis as a cause of childhood morbidity has led to renewed efforts at developing drugs for cryptosporidiosis.[10,410–412] Several groups have pursued high-throughput screening of pharmaceutical compound libraries, including some with licensed drugs and the Medicines for Malaria Venture library.[412] A piperaquine compound from the Medicines for Malaria Venture library (MMV665917) was highly effective in a calf model.[413,414] Malaria lipid kinase (MI4K8) was identified as a target for chemotherapy from a high-throughput screen. An inhibitor of MI4K8, KDU731, has proven effective in vitro, in small animal models, and in cattle.[415] The leprosy drug clofazimine was identified as active against *Cryptosporidium* by high-throughput screening.[416] A phase II study in AIDS patients with cryptosporidiosis is currently enrolling. A drug repurposing screen identified 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors as potential treatments, and atorvastatin significantly reduced oocyst shedding in immunosuppressed mice.[417,418]

Other groups have targeted specific parasite molecules.[10,412] For example, an inhibitor of cryptopain (a parasite cysteine protease) was able to control infection in an IFN-γ knockout mouse model.[10,419] Calcium-dependent protein kinases (CDPKs) of apicomplexan parasites have an active site that is more open than in mammalian enzymes.[420–422] A number of bumped kinase inhibitors have also demonstrated efficacy in vitro without inhibiting mammalian kinases. Some have efficacy in small animal models and in cattle. However, so far all have stopped short of clinical trials due to unanticipated adverse effects. Although some of these compounds have worked in large animal models, only clofazimine has entered clinical trials, but so far there is no proof of efficacy in people.

PREVENTION

Cryptosporidiosis is transmitted from person to person and via contaminated water and food. Water purification is an important public health measure. Because chlorination has little effect on the oocysts, water purification should generally involve flocculation and filtration.[46,97,423] As noted, enhanced screening and improved water treatment have dramatically decreased the number of outbreaks associated with drinking water in the United States and United Kingdom.[43,46,110] Ultraviolet radiation or ozonation can disinfect contaminated water, but these methods are rarely used. Recreational waters, such as lakes, may pose a danger for compromised hosts, who should avoid untreated water.[424] Swimming pools and other recreational aquatic facilities are now an important source of infection, and anyone with diarrhea should not swim in public facilities.[116,286,425,426] Contamination of treated recreational water, such as a fecal accident in a swimming pool, should prompt aggressive measures, including closing the pool temporarily.

Personal measures can be used to decontaminate infected or potentially infected water, such as during travel to resource-poor countries when the public water supply is contaminated, or as a routine practice in compromised hosts. Water can be decontaminated by bringing it to a boil or by using a filter with a pore size of 1 μm or smaller.[427] Studies in resource-limited settings have demonstrated inconsistent effects of water treatment. Use of ceramic filters has decreased diarrheal diseases in some settings, but had mixed results in cryptosporidiosis.[428,429] Even providing bottled water did not decrease cryptosporidiosis in India.[430]

Although cryptosporidiosis can be transmitted within health care facilities, risk is minimal with standard precautions.[133] Gloves should be worn and hands washed after handling material contaminated with fecal material. Instruments such as endoscopes need to be carefully disinfected between uses. Wearing gloves and hand washing can also prevent infection in daycare centers. However, one outbreak did not abate until all of the infected children were treated with nitazoxanide.[128]

For patients with AIDS (CD4 cell counts <200 cells/μL), water should be boiled or filtered when water is contaminated.[424] If HIV-infected persons travel in resource-poor countries, they should be warned to meticulously avoid drinking tap water. Rifaximin is approved for prevention of traveler's diarrhea and has been used to treat some cryptosporidiosis cases.[431] However, there are no data on use of rifaximin to prevent cryptosporidiosis. These individuals should avoid obvious sources of *Cryptosporidium* oocysts, such as persons with diarrhea (particularly avoiding sexual practices that might involve exposure to feces), farm animals (particularly cattle), and domestic pets that have diarrhea. Chemoprophylaxis may also be considered but is generally not recommended. Two retrospective studies examined data from trials of prophylaxis of *M. avium* for their effects on cryptosporidiosis. Both noted a lower incidence of cryptosporidiosis in groups treated with rifabutin.[403,405] However, the incidence of cryptosporidiosis was low in both studies, such that chemoprophylaxis is not routinely recommended.

Experimental studies have suggested that it may be possible to develop a vaccine to prevent cryptosporidiosis.[10,222,432] However, current studies with recombinant vaccines have been less effective than priming by infection.[433] Vaccination would likely have to involve both human and animal hosts and would need to work against a number of species of parasites. Studies have so far not even demonstrated the feasibility of this approach.

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283 *Cyclospora cayetanensis, Cystoisospora belli,* Species, *Balantidium coli,* and *Blastocystis* Species

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SHORT VIEW SUMMARY

CYCLOSPORA AND *CYSTOISOSPORA*

Epidemiology
- Both are opportunistic pathogens in immunocompromised hosts but also infect immunocompetent patients.
- *Cyclospora:* distribution is worldwide and is endemic in developing areas, with outbreaks in developed areas.
- *Cystoisospora:* occurrence is primarily in tropical and subtropical climes, especially South America, Africa, and Southeast Asia.
- Contaminated food and water are primary sources.
- Oocysts can survive in the environment for months but must sporulate to become infective.

Diagnosis
- Acute or chronic diarrhea occurs with other constitutional and gastrointestinal symptoms.
- Oocysts in stool may be visualized using modified acid-fast stain.
- Multiple stool examinations may be required.

Therapy
- Trimethoprim-sulfamethoxazole (1 double-strength tablet twice daily for 7 to 10 days) or ciprofloxacin (500 mg orally twice daily for 7 days) is effective (see Tables 283.1 and 283.2).

SARCOCYSTIS SPECIES

Epidemiology
- Distribution is mainly tropical and subtropical, especially in Southeast Asia and Malaysia.
- Widely distributed in various animals; human disease is very rare and occurs after consumption of infected raw or undercooked animal flesh.
- *Sarcocystis suihominis* and *Sarcocystis hominis* are the main causes of human disease.
- *Sarcocystis nesbitti* has caused outbreaks of disease and may be associated with ingestion of water contaminated by snake feces.

Diagnosis
- Infection is generally asymptomatic.
- Rarely, self-limited gastrointestinal illness or myositis (fever, myalgias) occurs after exposure in endemic areas.
- Sporocysts or oocysts may be visible in microscopic examination of stool; muscle biopsy may be required.

Therapy
- None; albendazole has been used.

BALANTIDIUM COLI

Epidemiology
- Distribution is worldwide, with infections in Latin America, Southeast Asia, Papua New Guinea, and the Middle East most common.
- Pigs are primary reservoirs and shed cysts in stool.
- Cysts in contaminated food or water are infectious.

Diagnosis
- The infection is generally asymptomatic; occasionally a diarrheal illness is reported.
- Trophozoites are visible in stool.

Therapy
- Tetracycline, metronidazole, or iodoquinol is used (see Table 283.3).

BLASTOCYSTIS SPECIES

Epidemiology
- Distribution is worldwide, primarily in developing countries, but prevalence varies markedly.

Diagnosis
- Role in human disease is unclear; diarrhea, flatulence, and abdominal discomfort are the most commonly reported symptoms.
- Microscopic diagnosis is challenging; trichrome stain is the most sensitive.
- Polymerase chain reaction assay is more sensitive and specific, and is becoming more widely available.

Therapy
- Therapy is often unsatisfactory.
- Trimethoprim-sulfamethoxazole, metronidazole, or iodoquinol is used (see Table 283.4).

COCCIDIA OTHER THAN CRYPTOSPORIDIA

Cyclospora, Cystoisospora, and *Sarcocystis* are coccidian parasites belonging to the phylum Apicomplexa, family Eimeriidae. Coccidian protozoan infections are well recognized but still relatively uncommon causes of diarrheal disease. *Cryptosporidium parvum* and *Cyclospora cayetanensis,* two of the more commonly identified coccidian pathogens in diarrheal illness, account for a small but increasing proportion of laboratory-confirmed diarrheal disease reported to the Foodborne Diseases Active Surveillance Network (FoodNet) of the US Centers for Disease Control and Prevention. In 2011, the reported incidences of cryptosporidiosis and cyclosporiasis were 2.85 and 0.05 per 100,000 population, respectively[1]; in 2017, these rates increased to 3.7 and 0.3 per 100,000 population (1836 and 163 cases), respectively.[2] The almost fivefold increase in cyclosporiasis incidence may reflect, at least in part, changes in testing practices as well as increased use of culture-independent tests (e.g., molecular tests). However, these data need to be put into perspective when statistics show that *Campylobacter* and *Salmonella* infections occurred at rates of 16.0 and 19.1 per 100,000, respectively, during the same year.[2]

Cyclospora species are genetically closely related to *Eimeria* and are more distantly related to *Cystoisospora, Sarcocystis,* and *Toxoplasma.*[3] Although several species of *Cyclospora* have been identified, humans are the only known hosts for *C. cayetanensis.* Humans are also the only recognized hosts for *Cystoisospora belli,* and no other *Cystoisospora* species has been confirmed to infect humans.

Clinical signs and symptoms do not distinguish disease caused by *Cyclospora, Cryptosporidium,* microsporidia, *Cystoisospora,* or other noninflammatory causes of diarrhea. However, knowledge of endemic regions, the occurrence of global outbreaks, and the seasonal and geographic variation of diseases such as cyclosporiasis can help point to a particular pathogen. Infection with any of these agents can cause protracted and severe illness in immunocompromised hosts, in particular those with human immunodeficiency virus (HIV) infection, although

the incidence of many coccidian infections has decreased since the introduction of highly active antiretroviral therapy.[4,5] *Cyclospora* continues to be implicated in foodborne and waterborne outbreaks of diarrheal disease. In contrast, *Sarcocystis* infection is typically asymptomatic and rarely causes gastrointestinal symptoms.

Cyclospora

Cyclosporiasis was first described in humans in Papua New Guinea in 1977. The organism was considered to be a blue-green alga but eluded accurate taxonomic classification until 1993, when Ortega and colleagues[6] in Peru succeeded in inducing sporulation and thus confirmed its genus, *Cyclospora.* It was named *C. cayetanensis* after the Universidad Peruana Cayetano Heredia in Lima, Peru, a major site of research on the infection.

Life Cycle

Cyclospora oocysts are spherical, measuring 8 to 10 µm in diameter. Ultrastructural studies of the unsporulated oocyst reveal an outer fibrillar coat and a cell wall and membrane. Unsporulated oocysts are excreted in the stool of infected individuals. Oocysts are quite resistant and can survive under diverse environmental conditions, including freezing, 2% formalin, 2% potassium dichromate, and chlorination. Sporulation is required for infectivity and requires at least 7 days of maturation outside the human host; experimentally, in moderate temperatures, sporulation occurs within 7 to 13 days.[7] Each sporulated oocyst contains two sporocysts that each hold two sporozoites.

After ingestion of sporulated oocysts, excystation occurs in the proximal small bowel. Sporozoites penetrate the epithelial cells of the small intestine, where both asexual and sexual reproduction take place. Although the asexual life cycle can continue endogenously within the intestinal epithelium, sexual reproduction leads to the development of zygotes. Zygotes mature into oocysts within the intestinal epithelium, which in turn are released into the stool after causing rupture of the host cells.

Epidemiology

Cyclospora infections occur worldwide, sporadically and in clusters, with a major increase in reported cases after its widespread recognition in the mid-1990s.[8] Cases have been reported from all regions of the world. The majority have been described in developing countries of the tropics and subtropics, where the disease seems to be endemic; sporadic cases of disease occur commonly in underdeveloped areas. Prevalence studies in stool samples from developed countries have identified *Cyclospora* in no more than 0.5% of samples.[8] Cases in developed nations tend to be more frequently associated with recognized waterborne and foodborne outbreaks. Outbreaks in North America in the early and mid-1990s—notably, one outbreak among employees of a Chicago hospital that was attributed to ingestion of water from a contaminated water storage tank[9] and a more widespread outbreak throughout the United States and Canada associated with consumption of contaminated raspberries imported from Guatemala[10]—brought considerable attention to this organism. Other produce, including lettuce, basil, cilantro, watercress, and sugar snap and snow peas, has been implicated in North American foodborne outbreaks since 2000.[11–14] Produce is presumably contaminated by being washed or sprayed with contaminated surface water.[8,15] *Cyclospora* can also cause traveler's diarrhea, although it is not one of the major causes of this illness. Travelers accounted for 37% of all cyclosporiasis cases reported to FoodNet in 2015, but only 1.3% of all travel-related enteric infections were attributed to *Cyclospora* in the same year.[16] Cyclosporiasis is a recognized opportunistic infection in those with HIV infection and other immunosuppressed conditions.

Humans are the only known hosts for *C. cayetanensis.* Transmission occurs via the fecal-oral route. The risk for transmission and infection depends on the level of sanitation, as well as the availability of water and food that are at risk of being contaminated. Direct person-to-person spread is unlikely, owing to the need for oocysts to sporulate to become infectious. The infectious dose has not been determined but is presumed to be low. In developing countries infection is more common in children younger than 10 years of age,[17–19] with the risk for infection decreasing with increasing age. Infants may be somewhat protected through breastfeeding and the absence of exposure to environmental sources of the parasite. Infection occurs seasonally but varies according to geography, with the highest incidence in spring and summer (May through July) in Canada and the United States, in the warm season (April through June) in Peru,[17] before and during the monsoon season (May through October) in Nepal,[20] during drier months (January through March) in Haiti,[21] and during the rainy season (May to August) in Honduras.[22] Factors affecting seasonality and possible reservoirs during the off-season have not yet been defined. Multilocus sequence (genetic) typing suggests that there are geographic differences in circulating subpopulations of *C. cayetanensis,*[23] which could potentially benefit outbreak investigations by facilitating identification of the source(s) of an outbreak.

Cyclospora cayetanensis–like oocysts have been recovered from a variety of other animals, including mice, rats, dogs, chickens, ducks, and nonhuman primates. Attempts to infect mammals and birds in the laboratory setting have been largely unsuccessful. It is unclear what if any role animals play in the spread of infection and whether oocysts recovered from animal feces represent coprophagy or other zoonotic organisms that resemble *Cyclospora.* Oocysts have also been identified in sewage and vegetable washings.[15]

Clinical Manifestations

The clinical manifestations of *Cyclospora* infection are varied and differ according to age as well as the degree of endemnicity of the region in which infected individuals live. Asymptomatic infection is more common in the indigenous populations of developing countries, particularly in adults but also in children, suggesting that previous exposure may induce some degree of protective immunity among residents of these regions.[17,24] However, asymptomatic infection may also occur in others, including those with HIV infection.

Symptomatic disease occurs in both endemic and nonendemic regions. In developing countries, symptomatic disease is more likely to develop in the absence of previous exposure and is thus more common in children. After an incubation of 1 to 11 days (mean, 7 days), illness begins abruptly. A flulike illness may precede the onset of diarrhea, which is invariably present with a median of 6 (range, 5 to 15) watery stools per day.[25] Fatigue, anorexia, myalgia, abdominal cramps, flatus, and nausea occur frequently. Fever is present in approximately 25% of cases. Illness generally lasts from 1 to 7 weeks or longer and may result in dehydration and significant weight loss. Diarrhea can be cyclic or relapsing, especially in the absence of therapy. Disease may be severe in the elderly and life threatening in the immunocompromised; diarrhea and weight loss tend to be more severe, and illness can be prolonged, in individuals with acquired immunodeficiency syndrome (AIDS). Postinfectious fatigue can be profound in some individuals and may persist long after the resolution of other clinical symptoms. It is unknown whether the pathogenesis of disease is due to enterocyte dysfunction or whether toxins are secreted.

Extraintestinal complications of *Cyclospora* infection are exceedingly uncommon. Reactive arthritis[26] and Guillain-Barré syndrome[27] have both been reported after infection with *Cyclospora.* Biliary tract disease has been described in patients with AIDS.[28]

Diagnosis

The diagnosis of cyclosporiasis generally relies on the microscopic identification of oocysts in stool samples (Fig. 283.1A). Shedding of oocysts in stool can precede the onset of clinical illness, but the disappearance of symptoms and oocysts usually occurs simultaneously. Oocysts may be shed in low numbers during infection, and both concentration of stool specimens and collection of multiple specimens may be required to make the diagnosis.[8]

Although *Cyclospora* oocysts are approximately twice the size of *Cryptosporidium* oocysts, the two may be confused if oocysts are not measured. The organism is variably acid-fast on modified Ziehl-Neelsen or Kinyoun stain (see Fig. 283.1B), and such techniques are superior to the examination of routine wet mounts, which require a trained eye for identification of the organism. Therefore if cyclosporiasis is suspected, notification of the laboratory is prudent so that appropriate tests can be performed. Demonstration of blue autofluorescence of the oocysts

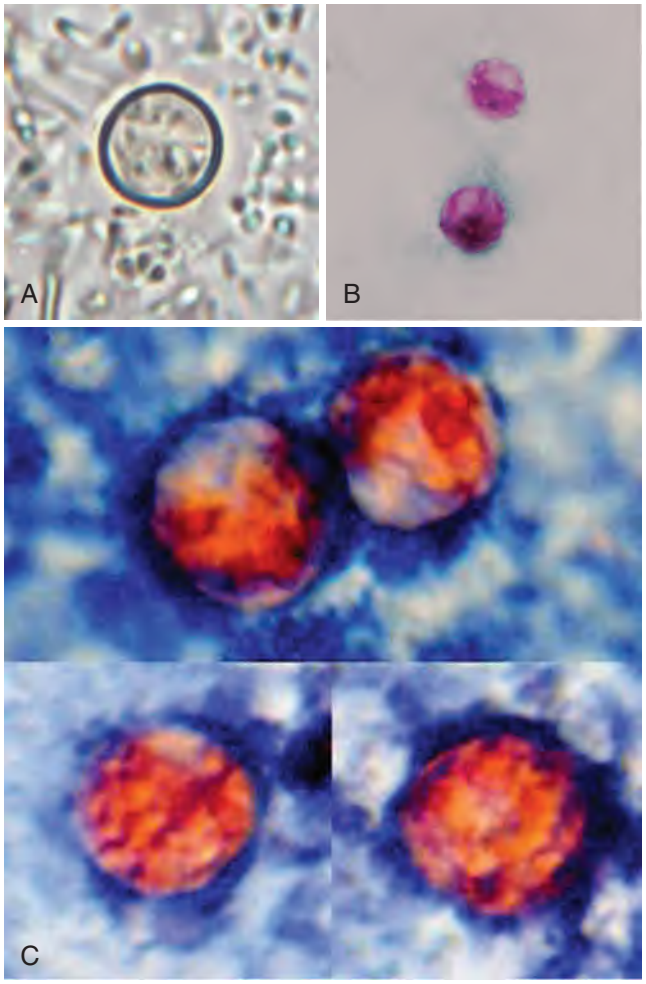

FIG. 283.1 *Cyclospora* oocysts visualized with different staining methods. (A) Wet mount. (B) Variable staining with modified acid-fast stain. (C) Uniform staining with modified safranin stain. Modification consists in heating in a microwave during staining. *(From DPDx Image Library, Centers for Disease Control and Prevention, Atlanta, GA.)*

under ultraviolet epifluorescence microscopy is both rapid and sensitive, although not specific. Additional stains, including auramine, safranin (see Fig. 283.1C), and lactophenol cotton blue, can also be used. Oocysts cannot be identified using Giemsa and trichrome stains.

Species-specific real-time polymerase chain reaction (PCR) assays have been developed that are capable of detecting low concentrations of oocysts in stool. Newer multiplex PCR-based diagnostic panels include *Cyclospora.* Although PCR assays may be more sensitive than conventional diagnostic methods,[29] they are costly and may lead to an increased number of diagnosed cases that in turn may require more expert decision making to interpret the results, particularly in endemic settings.[30] Flow cytometry has been proposed as an alternate method of diagnosis.[31] Antibodies to *Cyclospora* can be detected, but serologic tests are not commercially available.

The diagnosis may also be made by histopathologic or electron microscope examination of jejunal aspirates or biopsy specimens. Endoscopic findings may be normal or may demonstrate inflammation. Microscopic examination of tissue reveals altered histologic architecture of the small bowel, and loss of the brush border and altered epithelial cell morphology may be noted.[32] Villous atrophy, acute and chronic inflammation in the lamina propria, and vascular dilation may be seen. Routine hematoxylin and eosin staining of biopsy material may not permit adequate visualization of the organisms. Tissue sections may reveal *Cyclospora* in supranuclear locations within the cytoplasm, distinguishing them from *Cryptosporidium,* which are on the surface of enterocytes.

TABLE 283.1 Therapy (Adult) for Cyclosporiasis

DRUG[a]	THERAPEUTIC DOSAGE	PROPHYLACTIC DOSAGE
Trimethoprim-sulfamethoxazole	1 DS tablet[b] PO bid for 7–10 days	1 DS tablet PO 3 times weekly
Ciprofloxacin	500 mg PO bid for 7 days	500 mg PO 3 times weekly
Nitazoxanide	500 mg PO bid for 7 days	

[a]Drugs are listed in order of preference.
[b]One DS tablet contains 160 mg trimethoprim/800 mg sulfamethoxazole.
DS, Double strength; *PO,* orally.

Therapy

Trimethoprim-sulfamethoxazole (TMP-SMX) is the recommended therapy for cyclosporiasis (Table 283.1). One double-strength tablet (160 mg TMP/800 mg SMX) given twice daily is the usual dose for adults with normal renal function; for children, weight-based dosage (TMP 5 mg/kg twice daily) should be used. Treatment is continued for 7 days in immunocompetent hosts[17,33] and for 7 to 10 days in patients with HIV infection.[33,34] Eradication of oocysts correlates with treatment success. Suppressive therapy (TMP-SMX 160/800 mg three times weekly for 4 weeks) may be required in HIV-infected patients because of the historically high relapse rate (almost 50%) in this population.[34]

Individuals who cannot tolerate TMP-SMX may be treated with ciprofloxacin 500 mg twice daily for 7 days, based on a study conducted in HIV-infected patients.[35] If suppressive therapy is indicated, a dose of 500 mg three times weekly may be used. Ciprofloxacin was, however, slightly less efficacious than TMP-SMX for both treatment and prophylaxis.[35] The thiazolide agent nitazoxanide (500 mg twice daily for 7 days) has also been used successfully to treat cyclosporiasis in an immunocompetent adult[36] and was efficacious in the treatment of mixed parasitic infections (including cyclosporiasis) in children.[37] However, studies demonstrating its efficacy in the treatment of cyclosporiasis in HIV-infected patients are lacking.

Cystoisospora belli

Cystoisospora belli (formerly *Isospora belli*) was first described in 1915. It is the only one of more than 200 identified *Cystoisospora* species that is known to cause human infection. Human infections previously attributed to *Cystoisospora hominis* are more likely to have been caused either by *Sarcocystis* species or by misidentified *C. belli.*

Life Cycle

Immature *Cystoisospora* oocysts, each containing a single sporoblast, are excreted in the stool of infected hosts. Oocysts can remain viable in the environment for months. Sporulation in the environment is required before oocysts become infectious. Sporulation generally requires 24 to 48 hours but can occur within 16 hours in ideal conditions (30°C–37°C) and is hindered at temperatures below 20°C or above 40°C.[38] The single sporoblast divides in two, and each newly formed sporoblast subsequently matures into a sporocyst. The resulting infective elliptical oocyst (22–33 × 12–15 µm) contains two sporocysts, each with four sporozoites.

Ingestion of sporulated oocysts results in the release of sporozoites in the proximal small intestine. Sporozoites may develop into merozoites, with subsequent asexual reproduction occurring within enterocytes; over time, sexual reproduction follows, resulting in the development and passage of immature, unsporulated oocysts in feces. Rarely, sporozoites can migrate out of the intestine to various tissues, where they may remain dormant as cysts and later give rise to extraintestinal disease.

Epidemiology

Cystoisospora species are found worldwide but predominantly in tropical and subtropical climates, especially in South America, Africa, and Southeast Asia. *Cystoisospora* occasionally cause traveler's diarrhea. In the United

States, cystoisosporiasis has been more commonly associated with HIV infection and other immunosuppressed conditions, immigration from Latin America, daycare centers, and psychiatric institutions. In patients with AIDS in the United States, *C. belli* infection accounted for 2% to 3% of AIDS-defining illnesses in the 1980s, but this decreased to less than 0.1% in the late 1990s,[39] largely because of the widespread use of TMP-SMX to prevent *Pneumocystis jirovecii* pneumonia. A recent systematic review of *Cystoisospora* in HIV infection identified a global pooled prevalence of 2.5%, with a higher prevalence in Africa and southeast Asia.[40]

Cystoisospora species other than *C. belli* have been found in a wide variety of animals, including cats and dogs. It is unclear whether most animals develop clinical disease or whether they merely act as paratenic hosts. Pigs are notable exceptions; *Cystoisospora suis* can cause severe diarrheal disease and death in piglets and has been implicated in outbreaks of disease among nursing piglets.[38]

Clinical Manifestations

The pathogenesis of cystoisosporiasis has not been determined but may be the result of cell damage from direct consequences of parasite invasion, cell-mediated inflammation, or proteins and oxidants released from mast cells.

In immunocompetent hosts, *Cystoisospora* infection is indistinguishable from other noninflammatory intestinal infections. After an incubation period of approximately 1 week, a self-limited diarrheal illness usually develops that lasts 2 to 3 weeks and is characterized by malaise, anorexia, weight loss, abdominal cramps, and profuse watery diarrhea without blood. Fever is uncommon and if present is usually low grade. Oocyst shedding may persist for several weeks after recovery. Rarely, chronic persistent or intermittent symptoms may continue for many years. Biliary tract disease due to *Cystoisospora* has been described in immunocompetent hosts.[41]

In immunocompromised hosts, including those with HIV infection or malignancy and those receiving cytotoxic therapy, infection may result in protracted, severe diarrheal illness. Cystoisosporiasis in HIV-infected patients generally occurs with CD4 T-cell counts less than 200 cells/mm.[3] Hemorrhagic colitis,[42] biliary tract disease,[43] disseminated disease,[44] and reactive arthritis[45] have been reported in the literature.

Diagnosis

Typically, *C. belli* infection is diagnosed by identification of oocysts in stool in wet mounts (Fig. 283.2A) or modified acid-fast–stained fecal smears (see Fig. 283.2B). Because *C. belli* parasites are shed intermittently in low numbers, multiple stool examinations may be required for diagnosis, and stool concentration (using flotation or sedimentation methods) may be required. Direct or concentrated wet mounts are preferable to permanent stain smears because oocysts are difficult to detect in preserved stool specimens. Auramine-rhodamine, lactophenol cotton blue, and safranin (see Fig. 283.2C) may also be used. Ultraviolet autofluorescence microscopy (see Fig. 283.2D) is a simple, rapid, and sensitive diagnostic method that is based on the detection of oocysts that autofluoresce (blue) when a 330- to 380-nm ultraviolet filter is used. Examination of small bowel specimens (e.g., duodenal aspirates) may be helpful if stool examination is negative. Peripheral blood eosinophilia and Charcot-Leyden crystals in stool, both unusual in other protozoan infections, have been reported.[46] PCR assay can identify *C. belli* with high sensitivity and specificity.

Histologic examination of the small bowel of infected patients is relatively nonspecific and reveals villous atrophy, crypt hyperplasia, and lamina propria infiltration with inflammatory cells, particularly eosinophils. Asexual and sexual stages of the parasite can be identified within parasitophorous vacuoles of enterocytes. In case reports of extraintestinal disease, intracellular cysts containing one to three trophozoites were identified in lymph nodes, liver, and spleen.[44] Gallbladder pathology in biliary disease reveals eosinophilic intraepithelial parasites within parasitophorous vacuoles, epithelial disarray, and intraepithelial lymphocytosis.[41]

Therapy

Drug therapy (Table 283.2) has been studied predominantly in HIV-infected patients. TMP-SMX (160 mg TMP/800 mg SMX), the treatment of choice, is administered two times daily for 10 days, although HIV patients may need 1 double-strength tablet four times daily for up to 4 weeks.[47] Patients with HIV infection usually respond to antimicrobial therapy within several days but have a 50% chance of relapse within 6 to 8 weeks if suppressive therapy is not administered.[48] TMP-SMX for this purpose is beneficial when taken either daily or three times weekly.[49]

Alternative antibiotics include ciprofloxacin (500 mg twice daily for 7 days followed by suppressive therapy three times weekly),[35] or

TABLE 283.2 Therapy (Adult) for Cystoisosporiasis

DRUG[a]	THERAPEUTIC DOSAGE	PROPHYLACTIC DOSAGE
Trimethoprim-sulfamethoxazole	1 DS tablet[b] PO bid for 10 days	1 DS tablet PO daily or 3 times weekly
Ciprofloxacin	500 mg PO bid for 7 days	500 mg PO daily or 3 times weekly

[a]Drugs are listed in order of preference.
[b]One DS tablet contains 160 mg trimethoprim/800 mg sulfamethoxazole.
DS, Double strength; *PO,* orally.

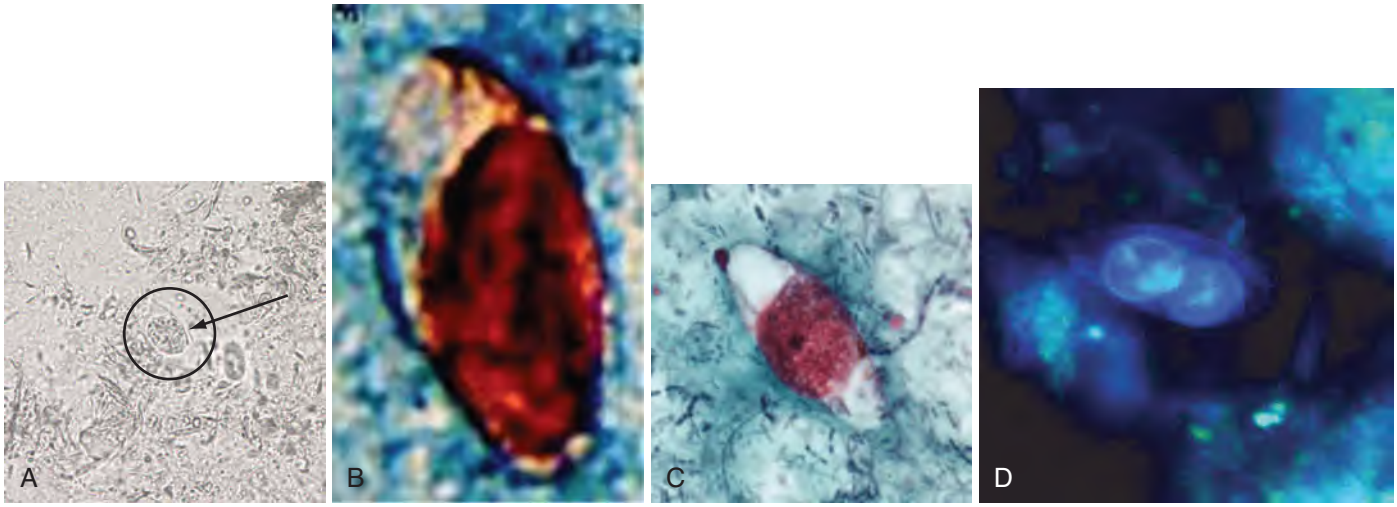

FIG. 283.2 Immature oocysts of *Cystoisospora belli,* each containing a single sporoblast. (A) Wet mount. (B) Modified acid-fast stain. (C) Stained with safranin. (D) Viewed under ultraviolet autofluorescence microscopy. *(From DPDx Image Library, Centers for Disease Control and Prevention, Atlanta, GA.)*