CRYPTOSPORIDIOSIS is caused by cryptosporidium, a parasite classified as an emerging pathogen by the Centers for Disease Control and Prevention (CDC). The organism infects the gastrointestinal epithelium to produce a diarrhea that is self-limited in immunocompetent persons but potentially life-threatening in immunocompromised persons, especially those with the acquired immunodeficiency syndrome (AIDS). Infection by this parasite accounts for up to 6 percent of all diarrheal disease in immunocompetent persons. The infection is also present in up to 24 percent of persons with both AIDS and diarrhea worldwide. A large water-borne outbreak in Milwaukee in 1993 affected an estimated 403,000 persons, 52 percent of those served by the contaminated waterworks. Despite the magnitude and severity of cryptosporidial infection, its pathogenesis is poorly understood, and there is currently no fully effective therapy.

THE PARASITE

Cryptosporidium is an intracellular parasite within the protist phylum Apicomplexa, group Alveolata. Ten species are currently recognized on the basis of differences in host specificity and oocyst morphology, and reclassification into a larger number of distinct species is anticipated. Although Cryptosporidium parvum is the most common species in humans, C. felis, C. muris, and C. meleagris have also been identified in immunocompromised persons. Two distinct C. parvum genotypes are known to infect humans: human type 1 and bovine type 2, with differences in the infectivity of various strains. C. parvum appears incapable of purine synthesis, relying on salvage pathways for hypoxanthine, guanine, and adenosine. Several biochemical pathways not present in mammalian cells have been identified in C. parvum, which provide unique parasite-specific targets for drugs.

Cryptosporidium is capable of completing all stages of its development (asexual and sexual) within a single host (Fig. 1). Humans are infected when they ingest cryptosporidium oocysts. Once ingested, oocysts excyst in the gastrointestinal tract and release infective sporozoites. In a process mediated by specific ligands on the sporozoite surface and receptors on the host cell, the sporozoite attaches to the apical membrane of the host epithelial cell. Such attachment induces reorganization of the host-cell actin cytoskeleton and protrusion of the host-cell membrane around the sporozoite to form a vacuole in which the organism remains intracellular but extracytoplasmic (Fig. 1B to 1F). At the base of each vacuole, an electron-dense band of host-cell cytoskeletal elements may facilitate the uptake of nutrients by the parasite from the host cell (Fig. 1E and 1F). The internalized sporozoite then matures and undergoes asexual reproduction (schizogony) to produce merozoites. After release into the intestinal lumen, merozoites can either infect other epithelial cells or mature into gametocytes, the sexual form of the parasite. The life cycle is repeated after fertilization occurs in the intestinal tract, yielding thin-walled oocysts that sporulate to release sporozoites again; this can lead to autoinfection and heavy, persistent infections, with massive shedding of oocysts in the feces of an infected patient.

EPIDEMIOLOGIC FEATURES

Since the first reported cases of human infection in 1976, cryptosporidiosis has become one of the most commonly reported enteric pathogens in both immunocompetent and immunocompromised persons worldwide. The proportion of the general population excreting oocysts is 1 to 3 percent in developed countries and 10 percent in developing countries. Cryptosporidial infection accounts for 2.2 percent (range, 0.26 to 22 percent) of cases of diarrhea in immunocompetent persons in developed countries and 6.1 percent (range, 1.4 to 41 percent) of cases of diarrhea in immunocompetent persons in developing countries. It occurs in up to 7 percent of children with diarrhea in developed countries and up to 12 percent of children with diarrhea in developing countries.
countries. Cryptosporidial infection is more common in immunocompromised persons, especially those with AIDS. In developed countries, it occurs in 14 percent (range, 6 to 70 percent) of patients with AIDS and diarrhea; in developing countries, it occurs in 24 percent (range, 8.7 to 48 percent) of such patients. The infection rate was 3 to 4 percent among patients with AIDS in the United States, but the rate fell after the introduction of highly active antiretroviral therapy. Cryptosporidiosis remains a clinically significant problem in patients without access to highly active antiretroviral therapy and in malnourished children, as well as in people in developed countries who have undergone transplantation or are receiving chemotherapy.

Persons at increased risk for cryptosporidial infection include the household and family contacts, as well as the sexual partners, of infected patients; health care workers; day-care personnel; users of communal swimming pools; and travelers to regions of highly endemic disease. Infection is frequently spread by person-to-person transmission, by animals, and indirectly through the environment (particularly by water). Most instances of human-to-human transmission occur directly by the fecal–oral route or indirectly by fomites, including sputum and vomitus. Zoonotic transmission from cattle and sheep to humans is known, and these animals are currently considered the most important reservoirs of human disease.

Cryptosporidial oocysts may be found in all types of water, including untreated surface water, filtered swimming-pool water, and even chlorine-treated or filtered drinking water. Contamination of untreated surface water and filtered public water supplies is a growing concern, since water-borne outbreaks have been reported worldwide. The 1993 outbreak in Milwaukee resulted in the death of several immunocompromised patients and illness in many previously healthy people. Outbreaks can also be caused by contamination of food and of water in swimming pools and sprinklers. The biologic and epidemiologic features of this parasite are summarized in Table 1.

**IMMUNOLOGIC ASPECTS**

Both humoral and cell-mediated immunity are involved in the resolution of cryptosporidiosis and resistance to infection. The invasion of epithelial cells in vitro by *C. parvum* results in the rapid expression of inflammatory chemokines; this response is important in the early development of the inflammation often observed in *C. parvum* infection. Increased production of prostaglandin E₂ (which stimulates mucus production) or the antimicrobial peptides β-defensins in *C. parvum*-infected cells helps to protect the epithelium from parasitic invasion. Interferon-γ is important for resistance to *C. parvum*, because the absence of this cytokine in both gene-knockout mice and humans with interferon-γ deficiency results in a high level of susceptibility to *C. parvum* infection.

Acquired resistance to cryptosporidial infection is dependent on T cells with the α/β type T-cell receptor; in addition, the CD4+ T-cell subgroup has a protective role, whereas γ/δ+ or CD8+ subpopulations of T cells appear irrelevant or subordinate. Specific IgG, IgM, IgA, and even IgE antibodies occur in serum from patients with acute infection or from convalescent patients. However, the mechanisms by which *C. parvum*-infected gastrointestinal epithelial cells elicit host immune responses are not understood; one possible mechanism in human cells involves the production of cytokines and chemokines by infected mucosa, with M cells in the intestine having a role.

**PATHOPHYSIOLOGIC FEATURES**

The proportion of exposed persons in whom cryptosporidiosis develops depends on both the infectivity of the parasite and the host immune response. Cryptosporidium appears not to infect tissue beyond the most superficial surface epithelia. There is a range of histologic abnormalities in crypt and villous structure, including villous atrophy and crypt hyperplasia, usually accompanied by a mixed inflammatory-cell infiltrate within the lamina propria. Cryptosporidium-induced diarrhea is associated with impaired intestinal absorption and enhanced secretion (Fig. 2). *C. parvum*-associated apoptotic epithelial-cell death has been identified in cul-

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**Figure 1 (facing page).** Life Cycle of Cryptosporidium and Infection of Host Epithelial Cells.

After being ingested, sporozoites are released, attach to the epithelial microvillous border, invade cells, and form trophozoites. Panel A through E show a *Cryptosporidium parvum* sporozoite attaching to and involving a host epithelial cell in an in vitro model of biliary cryptosporidiosis. Panels A, B, C, and D are scanning electron micrographs, and Panels E and F transmission electron micrographs. Panel A shows a sporozoite attaching to the apical membrane surface of a biliary epithelial cell. Panels B and C show a sporozoite invading a host cell and the protrusion of the epithelial-cell membrane around the parasite at its attachment site. Panels D and E show an organism being enveloped by the host-cell membrane and the formation of a vacuole. In Panel E, the zoite has made contact with the microvillus border of the epithelial cell, with its anterior end inserted into the host-cell membrane (arrow), and is in the process of being internalized. A dense band is formed where the parasite meets the epithelial cell. Panel F shows an intestinal-biopsy specimen from a patient with the acquired immunodeficiency syndrome and intestinal cryptosporidiosis. The bar represents 1 μm. The illustration of the life cycle is modified from Tzipori and Widmer, with the permission of the publisher.

Panels A, B, C, and D are reprinted from Chen et al., with the permission of the publisher.
tured intestinal and biliary epithelial cells and has been confirmed by limited clinical observations (Fig. 3B).\textsuperscript{16,32-34}

The molecular mechanisms by which cryptosporidium causes disease are unknown. An enterotoxin-like activity has been detected in fecal extracts, which may cause abnormal absorption and secretion and impaired epithelial permeability, but no enterotoxin has been purified (Fig. 2).\textsuperscript{28} Specific attachment to the apical surface of epithelial cells by the \textit{C. parvum} sporozoite, as well as molecules inserted into host cells after its attachment,\textsuperscript{5} appears to activate secondary signal pathways in the host cell, thereby altering cell function.\textsuperscript{23,35,36} \textit{C. parvum} also activates the nuclear factor-κB (NF-κB) system in directly infected biliary epithelial cells.\textsuperscript{37} Release of NF-κB–associated cytokines and chemokines has a critical role in the pathogenesis of inflammation associated with cryptosporidiosis.\textsuperscript{22,34} \textit{C. parvum}–induced epithelial-cell apoptosis in biliary infection is limited to nearby uninfectected cells and appears to be associated with the Fas receptor–Fas ligand death pathway.\textsuperscript{32} Thus, it appears that \textit{C. parvum} possesses a complex virulence capacity to invade epithelial cells and induce survival signals (e.g., NF-κB) in the infected cells, so that the organism can propagate, while simultaneously triggering alterations (e.g., apoptosis) in uninfected neighboring cells to impair the absorptive and secretive functions of epithelial cells, thus causing disease. Although human immunodeficiency virus type 1 (HIV-1) infection is not a prerequisite for cryptosporidium infection of host epithelial cells, there may be synergistic pathologic effects in response to dual infection. For example, recombinant HIV-1 tat protein, a peptide released in a biologically active soluble form from HIV-1–infected T cells and macrophages, enhances \textit{C. parvum}–associated apoptosis.\textsuperscript{28}

**CLINICAL FEATURES**

The intestinal tract is the primary site of cryptosporidiosis (Table 2). Although infection can be asymptomatic, most patients have profuse watery diarrhea containing mucus but rarely blood or leukocytes. The duration and severity of clinical symptoms depend largely on the immune status of the infected person. In immunocompetent persons, the disease is usually either asymptomatic or self-limited. The three major clinical presentations in immunocompetent persons are asymptomatic carriage, acute diarrhea, and persistent diarrhea that may continue for several weeks.\textsuperscript{28} After an incubation period of 7 to 10 days, more than 90 percent of infected patients present with acute watery diarrhea that lasts approximately 2 weeks, accompanied by nausea, vomiting, and cramp-like abdominal pain; 36 percent also have fever. In the outbreak in Milwaukee, the mean duration of illness was 12 days, and the median maximal number of stools per day was 12. The clinical manifestations included watery diarrhea (in 93 percent of patients), abdominal cramps (in 84 percent), fever (in 57 percent), vomiting (in 48 percent), and weight loss (in 75 percent).\textsuperscript{2} Acute and chronic diarrhea due to \textit{C. parvum} in children in the developing world is associated with malnutrition and high morbidity and mortality rates.\textsuperscript{28} The diarrhea also has lasting adverse effects on weight and height.\textsuperscript{90,40}

The severity and duration of diarrhea and the extraenteric manifestations of the infection differ in

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**TABLE 1. BIOLOGIC AND EPIDEMIOLOGIC FEATURES OF \textit{CRYPTOSPORIDIUM PARVUM} THAT FAVOR TRANSMISSION TO HUMANS.**

<table>
<thead>
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<th>Feature</th>
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<tr>
<td>Outbreaks of water-borne infection occur.</td>
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<td>Organism has ubiquitous geographic distribution and wide host range.</td>
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<tr>
<td>Oocysts are resistant to disinfectants.</td>
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<tr>
<td>Transmission can occur by direct fecal–oral (person-to-person or zoonotic) route.</td>
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<tr>
<td>As few as 10 to 100 oocysts can cause infection.</td>
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<tr>
<td>Oocysts are excreted in fully infective form with no external maturation required and with the capacity to complete their entire life cycle in a single host.</td>
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<tr>
<td>Oocysts are excreted in very large numbers, which are increased by autoinfective stage of production.</td>
</tr>
<tr>
<td>Many asymptomatic infections exist.</td>
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<tr>
<td>Specific and fully effective treatment is lacking.</td>
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</tbody>
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*Modified from Meinhardt et al.\textsuperscript{20}*

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Cryptosporidium parvum attaches to microvillous membrane

C. parvum induces secretion of 5-HT and PGE₂

Enterotoxin released

Interleukin-8 triggers inflammatory reaction

Anti-apoptotic signals

Neutrophil

C. parvum induces secretion of 5-HT and PGE₂

Epithelial cells of ileum

Villous atrophy

Malabsorption

NF-κB c-src

Apoptotic cell death

Soluble factors such as tat protein

Human immunodeficiency virus type 1

T cell

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immunocompetent and immunocompromised patients. Patients with AIDS who have cryptosporidiosis have a wide spectrum of disease, from asymptomatic shedding of cryptosporidial oocysts to a fulminant cholera-like illness, depending on the site of cryptosporidial infection and the CD4+ T-cell count (Table 2). The four clinical patterns of disease in patients with AIDS are asymptomatic infection, in which patients have no change in bowel habits and pass fewer than three stools per day (4 percent of patients); transient infection, in which diarrhea lasts for less than two months and is followed by a complete remission of symptoms and loss of cryptosporidium from fecal specimens (29 percent); chronic diarrhea lasting two months or more, with persistence of the parasites in stool or in biopsy specimens (60 percent); and fulminant infection, in which patients pass at least 2 liters of watery stool daily (8 percent). Fulminant infection occurs only in patients with a CD4+ T-cell count below 50 per cubic millimeter, whereas transient or asymptomatic infection is associated with higher CD4+ counts. The diarrhea in patients with AIDS is usually watery, and the stool frequency can be up to 10 per day; these patients can have a 10 percent drop in body weight and usually have severe malabsorption. Most patients never clear the infection, and they have a shorter survival than patients with AIDS who do not have cryptosporidiosis.

Extraintestinal cryptosporidiosis has been reported principally in patients with AIDS. It may involve the lungs, middle ear, biliary tract, pancreas, and stomach. These sites probably represent extensions of a primary intestinal infection. Biliary cryptosporidiosis is the most common extraintestinal manifestation of infection. It was described in up to 26 percent of patients with AIDS and intestinal cryptosporidial infection in the era before highly active antiretroviral therapy. The true frequency of this entity is difficult to determine, because invasive procedures are required for diagnosis. The clinical features include pain in the right upper quadrant, nausea, vomiting, and fever, usually accompanied by elevated serum alkaline phosphatase levels. Those with biliary symptoms have lower CD4+ T-cell counts. A biliary reservoir of cryptosporidium may contribute to the chronic nature of the infection and the inability of therapy to eradicate the organism. Although biliary cryptosporidiosis increases morbidity in patients with AIDS, it may not affect survival. Respiratory involvement is rare and is characterized by cough, dyspnea, fever, and thoracic pain.

**DIAGNOSIS**

The diagnosis of cryptosporidiosis should be considered in all patients with acute or persistent diarrhea, especially if they are immunocompromised. The definitive diagnosis requires microscopical detection of the parasite in tissues or body fluids. However, clinical, endoscopic, immunologic, and molecular techniques all have a place in the diagnosis and clinical assessment of cryptosporidiosis. The simplest method of detecting oocysts is modified acid-fast staining.

![Figure 3. Biliary Cryptosporidiosis in Cholangiopathy in a Patient with the Acquired Immunodeficiency Syndrome (AIDS).](image-url)

Panel A shows a radiograph of secondary sclerosing cholangitis due to biliary cryptosporidiosis in a patient with AIDS. The biliary tree appears irregular and distorted, with dilatation and narrowing. Panel B shows associated epithelial-cell apoptosis in the biliary tract in a biopsy specimen of gallbladder from the same patient. Two organisms are adherent to the apical surfaces of the epithelial cells (arrowheads). Adjacent to the cells infected with Cryptosporidium parvum is an epithelial cell that is undergoing apoptosis, displaying morphologic evidence of nuclear condensation and fragmentation (arrow) (hematoxylin and eosin, ×400). Panel A is reprinted from Cockerill et al. and Panel B from Chen et al., with the permission of the publishers.
of the organism on microscopical examination of stool. The sensitivity and specificity of the test are improved by newer tools, such as immunofluorescent assays and antigen-capture enzyme-linked immunosorbent assays, which are now commonly used in diagnostic laboratories. Polymerase-chain-reaction–based techniques are available as research tests. Clinicians should realize that routine “ova plus parasite” examinations do not include tests for cryptosporidium, which need to be specifically ordered. Serologic tests are of limited value, because many healthy persons have antibodies to cryptosporidium.

When biliary disease is suspected, ultrasonography is the best initial diagnostic test. It will show thickening of the biliary-duct wall, dilatation of the gallbladder, or both. However, the most sensitive test for the diagnosis of biliary tract disease in HIV-1–infected patients is endoscopic retrograde cholangiopancreatography. If biliary disease is suspected and the patient has normal findings on ultrasound examination, endoscopic retrograde cholangiopancreatography should be considered. The most common cholangiographic pattern, which occurs in approximately 50 to 60 percent of patients, is papillary stenosis associated with intrahepatic sclerosing cholangitis (Fig. 3A). Other cholangiographic patterns include papillary stenosis alone; sclerosing cholangitis without papillary stenosis; and long, extrahepatic bile-duct strictures. Endoscopic ultrasonography is useful in detecting papillary stenosis and is superior to transabdominal ultrasonography in detecting associated abnormalities, such as dilatation and thickening of the wall of the common bile duct, and in ruling out associated conditions, such as stones, compression, and tumors. Although occasionally diagnostic, percutaneous liver biopsy is rarely helpful and has no important role in diagnosis.

**THERAPY**

The treatment of cryptosporidiosis is unsatisfactory. There is no antimicrobial chemotherapeutic agent that will reliably eradicate the organism. However, there are agents that appear to suppress infection. When highly active antiretroviral therapy reduces the HIV load, symptoms may resolve in patients with cryptosporidium infection. Some nucleoside antiviral agents have a direct effect on the growth of *C. parvum* in vitro. Because the clinical course of cryptosporidiosis depends largely on the immune status of the host, treatment options vary. Generally, asymptomatic and immunocompetent persons need no specific therapy. Supportive care with oral or intravenous fluids and electrolyte replacement helps correct the dehydration that accompanies acute diarrhea while the patient spontaneously recovers. In children, spiramycin may shorten the duration of oocyst excretion and diarrhea, although the data on this are conflicting.

In patients with AIDS, the best treatment is improvement of immune function with highly active antiretroviral therapy, which also helps resolve cryptosporidiosis infection. If highly active antiretroviral therapy is not possible or effective, combination therapy with an antimicrobial agent and an antidiarrheal agent will continue to be standard treatment for cryptosporidial diarrhea. Paromomycin, azithro-
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REFERENCES

POSTING PRESENTATIONS AT MEDICAL MEETINGS ON THE INTERNET

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