Cryptosporidium species: New Insights and Old Challenges

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Cryptosporidium species are protozoan parasites that cause mainly enteric illnesses in humans and other animals. The mode of transmission is most commonly waterborne, but other sources of infection, including foodborne and person-to-person spread, have been documented. The environmental form of the parasite is resistant to most water purification methods, including chlorination. Cryptosporidium infection usually causes a self-limited diarrheal illness but can be life-threatening in immunocompromised individuals. There is no effective therapy for cryptosporidiosis.

Cryptosporidium species are intracellular gastrointestinal parasites that were initially thought to cause disease only in animals [1]. The first case of cryptosporidiosis in a human was described in 1976 in a 3.5-year-old girl who developed self-limited enterocolitis [2]. The disease subsequently achieved widespread notoriety in the medical community during the early years of the AIDS epidemic and later after a massive outbreak in Milwaukee, Wisconsin, in 1993 [3]. The parasite continues to be a major threat to human health for 2 major reasons. First, the current methods of water purification are ineffective for its removal from the public water supply. Second, there is no effective therapy for cryptosporidiosis. Although most cases of cryptosporidiosis in humans are self-limited, the consequences of infection can be devastating in immunocompromised individuals and in children living in underdeveloped countries. Advances in our understanding of Cryptosporidium species have been limited because the organism can be propagated only in live animal hosts. Nevertheless, newer molecular techniques, such as PCR, have provided new insights into the complex epidemiology of this parasite.

EPIDEMIOLOGY

Cryptosporidium infection is a zoonosis with mainly bovine and human reservoirs. Humans are thought to acquire the parasite by the ingestion of oocysts, which are shed in the stool of infected animals or other humans. In developing countries, cryptosporidiosis is endemic and is one of the most common causes of persistent diarrhea among children. In the developed world, cryptosporidiosis mainly occurs in sporadic outbreaks and epidemics. Immunodeficient people, particularly those with deficiencies in cell-mediated immunity, are especially susceptible to Cryptosporidium infection and experience the most-severe consequences of this illness.

Cryptosporidium infection is an important cause of diarrhea in the developing world [4]. An extensive review of cases of diarrhea demonstrated that the incidence of Cryptosporidium infection among immunocompetent patients from developing countries was 6.1%. Children from underdeveloped countries appear to be at particular risk for Cryptosporidium infection: serological evidence of previous infection was present in ∼50% of children in rural China and nearly all children living in an urban slum in Brazil. In the United States, there have been occasional outbreaks of infection in day care centers, foodborne outbreaks, and outbreaks associated with recreational water (public swimming pools, lakes, and ponds). The majority of reported cases of cryptosporidiosis in humans have been associated with contaminated drinking water; the largest such outbreak occurred in Milwaukee in 1993 [3].

Cryptosporidium infection is probably underdiagnosed as a cause of community-acquired diarrhea in the United States. Approximately 2% of stools tested (presumably for active en-
teritis) are positive for Cryptosporidium species [5]. Furthermore, antibodies to Cryptosporidium have been detected in \( \sim 30\% \) of children and adults in the United States [6]. Even higher seroprevalence rates have been detected in certain populations, such as dairy farmers [6]. Patients with poorly controlled HIV infection and patients receiving immunosuppressive agents are at particular risk for cryptosporidiosis. Other causes of immunodeficiency, such as solid-organ transplantation or primary immunodeficiencies, x-linked hyper-IgM syndrome, severe combined immunodeficiency, selective IgA deficiency, and possibly IFN-\( \gamma \) deficiency, are also thought to increase the risk of infection with Cryptosporidium species [7].

**TRANSMISSION**

The best-documented routes of transmission are waterborne, foodborne, and person-to-person spread. The majority of the documented outbreaks of waterborne infection in the world have been attributed to contaminated drinking water supplies, although contaminated water used for recreational activities has also been implicated [5]. The first reported waterborne outbreak of cryptosporidiosis occurred in 1984 and was attributed to fecal contamination of a public artesian well in Texas. In the spring of 1993, the largest outbreak of waterborne disease of any kind recorded occurred in Milwaukee. More than 400,000 of \( \sim 1,600,000 \) people in the greater Milwaukee area developed cryptosporidiosis after consuming contaminated drinking water. The onset of illness correlated with an increase in the turbidity of the treated water from Lake Michigan, despite use of water purification methods that were standard at the time [3]. Although the source of contamination remains uncertain, recent genotypic analysis of 4 of the isolates indicates consistency with a human origin [8].

Unfortunately, despite the public attention garnered by this massive outbreak, there have been many subsequent outbreaks of Cryptosporidium infection attributed to contaminated drinking water in the United States and the United Kingdom. This is in part due to increased surveillance, but it also reflects our inability to rid the public water supplies of this troublesome parasite. Concern about the safety of the public water supply has prompted government authorities to issue standards for the detection of Cryptosporidium species. These guidelines have been updated as recently as 2001, and our ability to detect Cryptosporidium species in the public water supply has unquestionably improved [9].

However, although surveillance has improved, there is still no consensus between scientists and public officials about the safe limits of Cryptosporidium species in the water supply. Furthermore, implementation of practical, affordable, and effective methods for the elimination of the parasite from drinking water remains an elusive goal. The current strategy for elimination of Cryptosporidium species from the public water supply involves preventing contamination of water sources, physical removal of the organisms, and chemical or physical disinfection. However, these methods are only capable of reducing the number of oocysts, not eliminating the parasite from the water supply. Therefore, it is imperative that patients at risk for Cryptosporidium infection contact their local public water authorities for advice about the safety of their water supply. More sophisticated approaches, such as filtration, ultraviolet light irradiation, and ozone treatment, have not been widely applied for various reasons, including financial and health-related concerns [10].

Although waterborne routes of transmission have been the most notable, foodborne and person-to-person spread have also been documented. Cryptosporidiosis has been attributed to ingestion of contaminated apple cider, chicken salad, milk, and food prepared by an ill food handler [5, 11, 12]. Cryptosporidium species have also been detected in seawater and have even been found in commercially harvested oysters [5]. Another potential source of infection may be raw vegetables sold in the marketplaces in developing countries [5]. However, in the latter 2 cases, a causative relationship has not been established between environmentally detected Cryptosporidium species and cases of infection in humans. Nevertheless, although waterborne outbreaks of cryptosporidiosis in humans have been documented more frequently, foodborne transmission may be underappreciated. Finally, it should be mentioned that, although cattle are thought to be the most common animal reservoir of Cryptosporidium species, other species of animals, including reptiles, birds, and insects, have been shown to harbor the parasite [5]. It is not clear what role these other animal hosts have in the transmission of Cryptosporidium species.

**TAXONOMY**

Cryptosporidium species, like Cyclospora, Isospora, and Toxoplasma species, are members of the phylum Apicomplexa. Cryptosporidium species were initially distinguished by their size, host specificity, and site of infection within the animal. Recently, molecular techniques have been used to establish a genetic basis for these distinctions [13, 14]. There are currently thought to be 10 valid species of Cryptosporidium: Cryptosporidium andersoni, Cryptosporidium baileyi, Cryptosporidium felix, Cryptosporidium meleagris, Cryptosporidium muris, Cryptosporidium nasorum, Cryptosporidium parvum, Cryptosporidium saurophilum, Cryptosporidium serpentis, and Cryptosporidium wrairi. Cryptosporidium infections have been described in 152 different mammalian species. Although Cryptosporidium organisms are known to be zoonotic pathogens, human-to-human transmission seems to be the most common mode of transmission in the United States [5, 11]. Recent efforts to use PCR to amplify specific genetic loci...
have led to a much better understanding of the biological characteristics and transmission of the parasite. These studies have confirmed that *C. parvum*, the most common cause of cryptosporidiosis in humans, can be divided into 2 major genotypes. These 2 genotypes, human and bovine, are also referred to as genotype 1 and genotype 2, respectively [13]. With rare exceptions, *C. parvum* genotype 2 is capable of infecting humans and animals, whereas genotype 1 infects only humans [13]. This classification may be an oversimplification, however, because several recent studies have shown significant genetic polymorphisms between isolates of the same genotype. We and others have shown tremendous heterogeneity among genotype 1 isolates at the Cpgp40/15 (also known as “gp60/45/15”) locus, which encodes surface glycoproteins present on invasive stages of the parasite [15, 16]. Furthermore, these studies suggest that *Cryptosporidium* organisms may undergo genetic recombination within and between the 2 prototypal genotypes. Recently, Feng et al. [17] provided experimental evidence for genetic recombination after infection of a murine host with 2 different genotype 2 isolates via microsatellite analysis.

Although the clinical significance of these genotypes is not completely clear, some patterns are beginning to emerge. Most infections in humans appear to be caused by genotype 1 [5]. However, some studies have shown that genotype 2 infections are more common, particularly during the spring and in sporadic outbreaks in rural agricultural communities [11]. Infections in humans due to genotype 1 isolates have been reported to be associated with increased quantity and duration of oocyst shedding [18, 19].

Finally, it should be noted that other species and genotypes of *Cryptosporidium* in addition to *C. parvum* might infect humans. Several recent reports have described the isolation of *C. meleagridis*, *C. felis*, and the “dog” genotype from humans [5, 11]. The relative importance of these other species of *Cryptosporidium* in infections in humans is currently uncertain. Furthermore, because the taxonomy of *Cryptosporidium* is in a state of flux, the validity of some of the more unusual species may not endure.

**PATHOGENESIS**

Infection with *Cryptosporidium* begins when the ingested oocysts release sporozoites, which subsequently attach to and invade the intestinal epithelial cell. The parasite has a particular predilection for the jejunum and terminal ileum. This point deserves emphasis, because diagnostic evaluations, such as endoscopy, may miss the site of infection. In patients with AIDS, other sites within the gastrointestinal tract may be involved, including the stomach, duodenum, and colon, as well as the biliary tract [7]. The histopathological features of cryptosporidiosis include a minimal inflammatory infiltrate and blunting of the villus. More-pronounced inflammatory changes, such as disruption of the epithelial cell barrier and more-extensive infiltration of the lamina propria with inflammatory cells, are seen in immunodeficient patients [20].

The mechanism by which *Cryptosporidium* infection causes diarrhea remains elusive. The diarrhea is typically noninflammatory and is often profuse. The parasite does elicit a local inflammatory response, and increased production of prostaglandins and several cytokines, particularly IFN-γ, has been described [21]. It is possible that these inflammatory mediators may consequently alter solute transportation in the intestinal epithelial cell, leading to osmotic diarrhea. *Cryptosporidium* infection has also been shown to inhibit apoptosis in infected epithelial cells as well as promote it in adjacent epithelial cells in vitro [21]. This could theoretically prolong parasite survival and impair absorption in the intestinal mucosa. The presence of an enterotoxin has also been hypothesized but never conclusively demonstrated [21].

Whatever the mechanisms by which *Cryptosporidium* infection causes disease may be, attachment to and invasion of host cells are crucial primary events in pathogenesis. However, little is known about specific parasite and host molecules involved in these processes. Knowledge of such molecules is essential for understanding the pathogenic mechanisms used by this parasite. Increasing recognition of *Cryptosporidium* species as emerging pathogens in humans has led to the identification of proteins, including CSL, GP900, p23/27, TRAP C1, gp40/45, cp47, and gp15/Cp17, which are implicated in mediating these interactions [22]. However, progress in establishing the functional role of these proteins has been hindered by the inability to propagate *C. parvum* in vitro and the lack of suitable systems for genetic manipulation of the parasite.

**IMMUNE RESPONSE**

Cell-mediated immunity appears to be the major component of the immune response to *Cryptosporidium* infection. Although there is a prominent humoral response to infection, the exact role of antibodies in host defense to *Cryptosporidium* infection is unclear. The correlation between the decreased number of CD4⁺ T cells and the risk of *Cryptosporidium* infection is evident of the critical role that immune cells play in immunity [23]. Studies that use murine models in which CD4⁺ T cells were lacking confirmed the central role of these cells in adaptive immunity [23]. The cytokines IFN-γ, IL-12, and TNF-α have also been shown to be protective against *Cryptosporidium* infection in laboratory models [24]. CD4⁺ T cells appear to be the source of IFN-γ in the adaptive immune response, and these cells have been shown to limit experimental infection [23]. Innate immune mechanisms may also be important in resistance to *Cryptosporidium* infection, because mice
that lack adaptive immune cells are initially resistant to infection. Furthermore, mutations of the mannose-binding lectin gene, a component of the complement cascade, were associated with an increased risk of cryptosporidiosis in patients with AIDS [24]. Therefore, it seems that, although adaptive immunity is necessary for the resolution of cryptosporidiosis, innate immune mechanisms may be important in resistance to infection.

**CLINICAL MANIFESTATIONS**

Essentially all humans are susceptible to *Cryptosporidium* infection, although people with serological evidence of previous infection seem to be more resistant [25]. The parasite is highly infectious, with an ID$_{50}$ ranging from 9 to 1042 oocysts, depending on the isolate [11]. The presentation is highly variable but is typically characterized by watery diarrhea. The diarrhea is often accompanied by abdominal pain and occasionally systemic symptoms such as fever, malaise, nausea, vomiting, and loss of appetite. The symptoms begin $\sim$5 days after the ingestion of oocysts but can begin up to 2 weeks later. The duration of illness in immunocompetent hosts is variable, lasting from several days to 5 weeks [26]. It is worth noting that many exposed patients are asymptomatic. In a study of experimental infection in humans, one-third of the subjects who ingested oocysts had no diarrhea [27].

Although the disease is self-limited in most patients, in several other situations, cryptosporidiosis can cause significant morbidity and death. Before the widespread use of HAART, cryptosporidiosis was a relatively common opportunistic infection in patients with advanced HIV infection. The risk of acquiring cryptosporidiosis was correlated with lower numbers of CD4$^+$ T cells ($\leq$200 cells/mm$^3$) [7]. In patients with advanced HIV infection, the illness has a highly variable presentation. In a cohort of HIV-infected patients studied in Baltimore during 1985–1995, four patterns were described: transient infection, intermittent relapsing infection, cholera-like infection, and chronic infection [28]. Another well-described manifestation of *Cryptosporidium* infection in patients with AIDS is cholangitis, which is seen more often with more-severe immunosuppression, defined as a CD4$^+$ T cell count of $\leq$50 cells/mm$^3$. These patients have a poor prognosis and often present without the typical symptoms of abdominal pain [21]. Gastric cryptosporidiosis and pancreatitis have also been described in patients with AIDS. Other causes of immunodeficiency mentioned above have also been associated with these more recalcitrant forms of cryptosporidiosis [7].

Although *Cryptosporidium* infection does not have a predilection for any particular age group, it is common among children in developed and underdeveloped nations. Several published reports have shown that cryptosporidiosis may have devastating long-term effects in children in poorer countries. In Brazil, cryptosporidiosis and persistent diarrhea among children have been correlated with subsequent impairments in physical fitness and diminished cognitive function [29]. Sadly, it appears that these children with persistent diarrhea are caught in a vicious cycle of malnutrition, placing them at risk for further diarrheal illness, which in turn leads to progressively worse nutritional status [30].

**DIAGNOSIS**

Cryptosporidiosis can be diagnosed via invasive or noninvasive techniques. Intestinal analysis via biopsy with demonstration of intracellular forms of the parasite is specific, but the diagnosis may be missed, because most common sites of infection are less accessible endoscopically. The "gold standard" and perhaps most widely used test for the detection of *Cryptosporidium* oocysts in stool remains the modified acid-fast or Kinyoun stain (figure 1A). The test should be specifically requested, because it will not be performed as part of a routine examination for ova and parasites. Interpretation of the stained smear requires experience, because other organisms in the stool may stain acid fast. Several immunofluorescent assays (figure 1B) and EIA kits...
have become commercially available and show promising sensitivity and specificity. These tests use antibodies against *Cryptosporidium* antigens to detect the parasite in stool specimens. One of these kits, the ColorPAC *Cryptosporidium/Giardia* rapid assay (Becton-Dickinson), was recently recalled because of a cluster of false-positive results [31]. PCR-based techniques also accurately detect the parasite in environmental samples and stool specimens but have yet to be standardized for routine clinical use and are not yet commercially available.

**THERAPY**

There is no effective antimicrobial agent for treatment of *Cryptosporidium* infection in humans, and, consequently, the US Food and Drug Administration has not approved a medication for this purpose. Paromomycin and, recently, nitazoxanide have been reported to have some clinical efficacy. The results of clinical trials of these 2 drugs have been mixed. Azithromycin in combination with paromomycin appeared to have a demonstrable effect in one uncontrolled trial involving patients with AIDS who have *Cryptosporidium*. Finally, resolution of *cryptosporidiosis* has been demonstrated after reconstitution of the immune system when HAART is used to treat advanced HIV infection [21].

**PREVENTION**

Patients with underlying immune system weaknesses are at risk for the more severe complications of *Cryptosporidium* infection, as mentioned above. In the absence of effective, specific therapy against infection with this parasite, preventative measures are of great importance among this patient population. Such measures include extensive hand washing, avoiding direct contact with stool from animals or humans, avoiding the accidental ingestion of water used in recreational activities, and taking measures to ensure the safety of the drinking water. It should be noted that the quality of the local drinking water is regionally and seasonally variable. Local public health and municipal water authorities can provide specific information about the safety of the water supply. *Cryptosporidium* species can be removed from drinking water by either boiling for 1 min or by filtering the water through a filter with a pore size of \(<1\) µm. These recommendations are well summarized on the Centers for Disease Control and Prevention’s Web site (http://www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis/factsht_crypto_prevent_ci.htm#12).

**References**