# Cryptosporidiosis: An Emerging, Highly Infectious Threat

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*Cryptosporidium parvum,* a leading cause of persistent diarrhea in developing countries, is a major threat to the U.S. water supply. Able to infect with as few as 30 microscopic oocysts, *Cryptosporidium* is found in untreated surface water, as well as in swimming and wade pools, day-care centers, and hospitals. The organism can cause illnesses lasting longer than 1 to 2 weeks in previously healthy persons or indefinitely in immunocompromised patients; furthermore, in young children in developing countries, cryptosporidiosis predisposes to substantially increased diarrheal illnesses. Recent increased awareness of the threat of cryptosporidiosis should improve detection in patients with diarrhea. New methods such as those using polymerase chain reaction may help with detection of *Cryptosporidium* in water supplies or in asymptomatic carriers. Although treatment is very limited, new approaches that may reduce secretion or enhance repair of the damaged intestinal mucosa are under study.

An emerging infection comes to our attention because it involves a newly recognized organism, a known organism that newly started to cause disease, or an organism whose transmission has increased. Although *Cryptosporidium* is not new, evidence suggests that it is newly spread (in increasingly used day-care centers and possibly in widely distributed water supplies, public pools, and institutions such as hospitals and extended-care facilities for the elderly); it is newly able to cause potentially life-threatening disease in the growing number of immunocompromised patients; and in humans, it is newly recognized, largely since 1982 with the AIDS epidemic. *Cryptosporidium* is a most highly infectious enteric pathogen, and because it is resistant to chlorine, small and difficult to filter, and ubiquitous in many animals, it has become a major threat to the U.S. water supply. This article will focus on the recognition and magnitude of cryptosporidiosis, the causative organism and the ease with which it is spread, outbreaks of cryptosporidiosis infection, and its pathogenesis, diagnosis, and treatment.

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## Recognition and Magnitude of Cryptosporidiosis

First recognized by Clarke and Tyzzer (1) at the turn of the century and well known to veterinarians, *Cryptosporidium* was reported as a human pathogen in 1976 by Nime (2). From 1976 until 1982, seven cases of cryptosporidiosis were reported in humans, five of which were in immunosuppressed patients. Since 1982, cryptosporidiosis has been increasingly recognized as a cause of severe, life-threatening diarrhea in patients with AIDS as well as in previously healthy persons (3). Of the first 58 cases of cryptosporidiosis described in humans by 1984, 40 (69%) were in immunocompromised patients who contracted severe, often irreversible, diarrhea (lasting longer than 4 months in 65%); of these 40 patients, 33 (83%) had AIDS (4-6); 55% of the 40 immunocompromised patients died.

A review of 78 reports of more than 131,000 patients and more than 6,000 controls showed *Cryptosporidium* infection in 2.1% to 6.1% of immunocompetent persons in industrialized and developing countries, respectively, vs. 0.2% to 1.5% in controls (Table 1). A review of an additional 22 reports of nearly 2,000 human immunodeficiency virus (HIV)-infected persons showed *Cryptosporidium* infection in 14% to 24% of HIV-infected persons with diarrhea vs. 0% to 5% of HIV-infected controls without diarrhea (7). Seroepidemiologic

Table 1. Rates of *Cryptosporidium* infection among immunocompetent and HIV-positive persons in industrialized and developing areas[abc]

| | Patients with diarrhea | Controls without diarrhea |
|---|---|---|
| Immuno-competent | | |
| Industr. areas | 2.2%(0.26%-22%) [n=2232/107,329] | 0.2%(0%-2.4%) [n=3/1941] |
| Developing areas | 6.1%(1.4%-40.9%) [n=1486/24,269] | 1.5% (0%-7.5%) [n=61/4146] |
| HIV-positive | | |
| Industr. areas | 14%(6%-70%) [n=148/1074] | 0%(0%-0%) [n=0/35] |
| Developing areas | 24%(8.7%-48%) [n=120/503] | 5%(4.9%-5.3%) [n=5/101] |

[a]From 100 reports of 133,175 patients with diarrhea and 6,223 controls. [b]Ranges given in parentheses. [c]Data from reference (7).

studies suggest that 17% to 32% of nonimmunocompromised persons in Virginia, Texas, and Wisconsin, as well as nonimmunocompromised Peace Corps volunteers (before travel), have serologic evidence of *Cryptosporidium* infection by young adulthood. In contrast, more than half of the children in rural Anhui, China, had serologic evidence of cryptosporidial infection by 5 years of age, and more than 90% of children living in an impoverished area of Fortaleza, Brazil, had serologic evidence of cryptosporidial infection in their first year of life (Figure) (8-11).

## The Organism

Among protozoa, *C. parvum* is the major human pathogen that is also found in numerous mammals. It is slightly smaller than the murine *Cryptosporidium*, *C. muris*, and is also distinguished from the other *Cryptosporidium* species commonly seen in birds, turkeys, snakes, and fish. Infection begins when a person ingests chlorine-resistant, thick-walled oocysts (7). These hardy oocysts appear to be infectious, with an estimated $ID_{50}$ (from studies in humans) of one isolate containing only 132 oocysts (12). Infections may occur with ingestion of as few as 30 oocysts; some infections have occurred with just one oocyst (13).

When the oocysts reach the upper small bowel, the proteolytic enzymes and bile salts enhance the excystation of four infectious sporozoites, which enter the brush border surface epithelium and develop into merozoites capable of replicating either asexually or sexually beneath the cell membrane (but extracytoplasmically) in the brush border epithelial cell surface. Sexual stages combine to form new oocysts, some of which (perhaps 20% as thin-walled oocysts) may sporulate and continue infection in the same person, while others (thick-walled oocysts) are excreted. Although few organisms may enter through M cells, systemic infection essentially does not occur; the occasional biliary tract or respiratory tract infections in immunocompromised patients probably reached these sites through the lumenal surface.

## Cryptosporidiosis Outbreaks

Numerous well-documented outbreaks of cryptosporidiosis have occurred. Most of these often waterborne outbreaks have involved subtle problems in the flocculation and/or filtration process (17-21). These outbreaks culminated in the huge waterborne outbreak in Milwaukee, which was initially thought to be viral gastroenteritis, reported to the State Health Department on April 5, 1993, diagnosed on April 7, and followed by an advisory note that evening to the public to boil all drinking water (Table 2). This became the largest waterborne outbreak in U.S. history and affected an estimated 403,000 persons, thus constituting a 52% attack rate among those served by the South Milwaukee water works plant. Several immunocompromised patients died, and many previously healthy persons became ill. The mean duration of illness was 12 days with a range of 1 to 55 days, and the average maximum number of watery diarrheal stools was 19 per day at the peak of illness. While watery diarrhea was the predominant symptom among 93% of confirmed

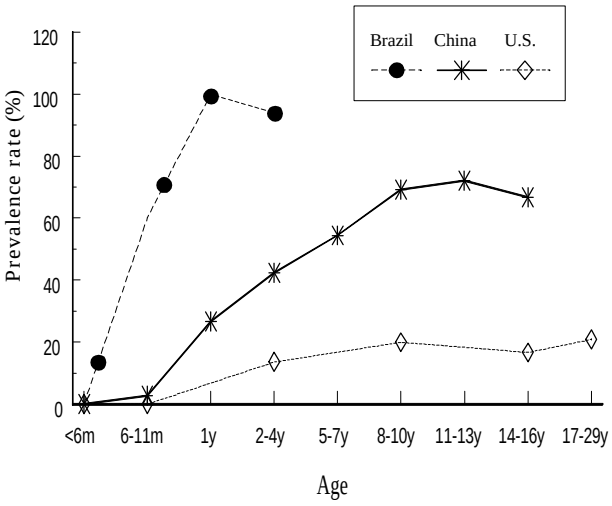


Figure. Prevalence of IgG antibodies to *Cryptosporidium parvum*, by age, in Brazil, China, and the United States.

Table 2. Symptoms of 205 patients with confirmed cases of cryptosporidiosis during the Milwaukee outbreak[a]

| Symptom | Percent(%) |
|---|---|
| Watery diarrhea<br>mean=12d; med=9d (1-55d)<br>mean=19/d; med=12/d (1-90)<br>39% recurred after 2d free | 93 |
| Abdominal cramps | 84 |
| Weight loss<br>(med=10lb, 1-40lb) | 75 |
| Fever<br>(med=38.3°, 37.2°-40.5°) | 57 |
| Vomiting | 48 |

[a]Data from reference (21)

cases, other symptoms such as abdominal pain, low-grade fever, and vomiting were not infrequent; 75% of infected nonimmunocompromised persons had an average 10-lb weight loss.

Additional outbreaks involving public swimming pools and wade pools have further documented the ability of *Cryptosporidium* to cause infection even when ingested in relatively small amounts of fully chlorinated water (22-26). While the leading causes of 129 drinking and recreational water outbreaks in the United States from 1991 through 1994 were *Giardia* and *Cryptosporidium*, cryptosporidiosis accounted for substantially more cases (even if the Milwaukee outbreak were excluded) (23,24,26). In addition, although *Cryptosporidium* oocysts cannot multiply in the environment, an outbreak of foodborne cryptosporidiosis, affecting 54% of those ingesting incriminated freshly pressed apple cider, has been reported (27). In this outbreak, *Cryptosporidium* oocysts were found in the cider press, as well as in a calf on the farm from which the apples were obtained. There was also a 15% secondary attack rate in households involved in this outbreak. The apparent person-to-person spread in households and institutions such as day-care centers and hospitals further documents the highly infectious nature of *Cryptosporidium*. In an urban slum area in northeastern Brazil, secondary household infections occurred in 58% of households with an infected child (index case) despite the 95% prevalence of antibody in children more than 2 years of age (28).

The spread of cryptosporidiosis in day-care centers is well documented, with 14 outbreaks reported in the United States, as well as others in the United Kingdom, France, Portugal, Australia, Chile, and South Africa (29). Illnesses usually occurred in the summer and early fall, especially during August and September in the United States and Portugal. Attack rates were 13% to 90%, with the highest rates found among nontoilet-trained toddlers and staff caring for children in diapers. Overall prevalence rates were usually in the 1.8% to 3.8% range; however, rates as high as 30% in day-care homes were reported (30). During outbreaks, 3.7% to 22.9% of infected children may not have diarrhea; infectious oocysts may be excreted for up to 5 weeks after diarrheal illness ends (31). In addition, numerous nosocomial outbreaks of cryptosporidiosis have occurred among health-care workers as well as patients in bone marrow transplant units, pediatric hospitals, and patient wards with HIV-infected patients (32-37). Furthermore, elderly hospitalized patients may also be at risk for *Cryptosporidium* infection (38). In one Pennsylvania hospital, 45% of nurses, medical students, and house staff caring for an HIV-positive patient with cryptosporidiosis seroconverted (39).

Numerous potential animal and water sources have been found to be infected with *Cryptosporidium*. In the Gonçalves Dias slum in Fortaleza, Brazil, 10% of animals (including dogs, pigs, donkeys, and goats), 6.3% during the dry season to 14.3% during the wet season, had *Cryptosporidium* in their stool specimens. In addition, 22% of drinking water sources studied were infected with *Cryptosporidium* oocysts (40). Furthermore, LeChavalier et al. have documented that *Cryptosporidium* oocysts were present in 27% of 66 drinking water samples obtained from 14 states and one Canadian province (mean of 0.18 NTU) (41,42).

## Pathogenesis and Impact

*C. parvum* does not infect tissue beyond the most superficial surface of the intestinal epithelium; however, it can derange intestinal function. Although a parasite enterotoxin has been extensively sought and some reports have suggested that one may exist (43), this issue remains controversial, and the source of substances in the stools of infected animals and patients that induce secretion remains unclear (44). Extensive studies in a piglet model of cryptosporidiosis by Argenzio and colleagues demonstrate the loss of vacuolated villus tip epithelium (approximately two-thirds of the villus surface area), accompanied by an approximate 50% reduction in glucose-coupled sodium cotransport. What remains is a predominance of transitional junctional epithelium, in which increased glutamine metabolism drives a sodium-hydrogen exchange, to which is coupled chloride transport. Thus, glutamine drives neutral

sodium chloride absorption in an apparent prostaglandin-inhibitable manner in *Cryptosporidium*-infected piglet epithelium (45). Furthermore, Argenzio and colleagues have demonstrated increased macrophages that produce increased tumor necrosis factor (TNF) in the lamina propria of *Cryptosporidium*-infected piglets (46). Although TNF did not directly affect epithelial transport, when a fibroblast monolayer was added, an indomethacin-inhibitable secretory effect was noted with TNF (46). Consequently, the researchers propose a prostaglandin-dependent secretory effect, which occurs 1) through a bumetanide-inhibitable chloride secretory pathway, predominantly from crypt cells; and 2) through the inhibition of neutral sodium chloride absorption through the amiloride-sensitive sodium:hydrogen exchanger, predominantly in the junctional or transitional epithelium during active cryptosporidial infection. Reduced xylose and B-12 absorption are among the effects described in humans and animals with cryptosporidiosis (47-49). Disruption of intestinal barrier function with strikingly increased lactulose to mannitol permeability and absorption has been documented during active symptomatic cryptosporidial infection in children and in HIV-infected adults (Lima et al., unpublished observations) (50).

*Cryptosporidium* appears to be one of the leading causes of diarrhea, especially persistent diarrhea, among children in northeastern Brazil (51,52). In addition, the incidence of diarrhea has been nearly double for many months in young children after symptomatic cryptosporidial infections, suggesting that the disrupted barrier function in infected children leaves residual damage resulting in increased susceptibility of injured epithelium to additional diarrheal illnesses (Agnew et al., unpub. obs.).

## Recognition and Diagnosis

The diagnosis of *C. parvum* in patients with diarrhea is usually made by using acid-fast or immunofluorescence staining on unconcentrated fecal smears. Appropriate concentration methods may enhance detection when small numbers of oocysts are present, but some methods such as formalin-ethyl acetate concentration may result in loss of many oocysts (52,53). While several enzyme-linked immunosorbent assay methods are available for detection of fecal cryptosporidial antigen with 83% to 95% sensitivity in diarrheal specimens, these methods are less sensitive in formed specimens and require more time. Microscopy using immunofluorescence antibody is slightly more sensitive and may be faster (54,55).

Polymerase chain reaction (PCR) provides a new method that may help detect *Cryptosporidium* in water supplies or asymptomatic carriers. A genomic DNA library has been constructed in the plasmid pUC18 for propagation in *Escherichia coli*. After sequencing a 2.3 kilobase *C. parvum*-specific fragment, a 400-base sequence with a unique *Sty* I site has been amplified by using primers of 26 nucleotides each (56). Laxer et al. then used a 20-base probe labeled with digoxigenin-11-dUTP to detect *C. parvum* DNA in fixed, paraffin-embedded tissue (57). In addition, primers for a 556 BP *Cryptosporidium*-specific region of the small subunit 18s ribosomal RNA gene have been used to produce a PCR product with unique Mae 1 sites that distinguish *C. parvum* from *C. baileyi* and *C. muris* (58). Available methods for detection of viable oocysts in environmental samples are relatively insensitive and under active investigation.

## Treatment and Prevention

Despite numerous attempts at examining transfer factor, hyperimmune colostral antibody, and more than 100 antiparasitic and antimicrobial agents, few agents have shown modest benefit in controlled studies; paromomycin is one of them. Although this agent does not eradicate the parasite in immunocompromised patients, it slightly reduces parasite numbers (from $314 \times 10^6$ to $109 \times 10^6$ oocysts shed per day) and decreases stool frequency, with a tendency toward improved Karnofsky scores and reduced stool weight (59). In view of its effectiveness in driving sodium cotransport (60) and its success in studies of experimental animals, we are examining a new approach to speeding repair of disrupted intestinal barrier function by using glutamine and its derivatives.

The ability of the thick-walled oocysts to persist and spread in the environment and their well-documented resistance to chlorine are responsible for the spread of *Cryptosporidium* even in fully chlorinated water supplies that meet existing turbidity standards in drinking water and swimming pools. Although some scientists have noted that 9,600 parts per million (mg/l) of chlorine for one minute of exposure are required to decontaminate water (14), others have noted that even after exposure to full-strength household bleach (5.25% sodium hypochlorite; 50,000 parts per million) for

2 hours, the oocysts still remained infectious for experimental animals (15). While *Giardia* are 14 to 30 times more susceptible to chlorine dioxide or ozone, respectively, ozone is probably the most effective chemical means of inactivating *Cryptosporidium* oocysts (16). Consequently, eradication of the organism from drinking water supplies depends on adequate flocculation and filtration, rather than chlorination. Although previous turbidity requirements were based on the removal of larger parasite cysts such as those of *Giardia lamblia* or *Entamoeba histolytica*, the smaller *C. parvum* oocysts are more difficult to remove. Several waterborne outbreaks, including the recent outbreak in Milwaukee, have occurred with turbidity levels in the 0.45 to 1.7 nephelometry turbidity units (NTU) range. In a study of waterborne cryptosporidiosis, predominantly among HIV-positive adults in Clark County, Nevada, Goldstein et al. (1996) report that the outbreak was associated with a substantial number of deaths and that the turbidity of the implicated water never exceeded 0.17 NTU (much lower than the new standard of 0.5 NTU required for 95% of measurements each month, with no spikes over 1.0 NTU) (5).

New approaches to the eradication of infectious oocysts from water supplies are needed, possibly using reverse osmosis, membrane filtration, or electronic or radiation methods, instead of the ineffective chemical or difficult filtration techniques currently used. Ideally, these new methods would provide low cost, effective treatment that could be applied in developing areas as well. Meanwhile, an improved understanding of the pathogenesis and impact of *Cryptosporidium* infections should aid the development of improved treatment and control of this ubiquitous, highly infectious threat to the water supply and to the people it serves, especially malnourished children and immunocompromised patients around the world.

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Dr. Guerrant is Thomas H. Hunter Professor of International Medicine and director, Office of International Health, University of Virginia School of Medicine. He holds several patents on innovative approaches to the diagnosis and treatment of common gastrointestinal illnesses. In addition to his many other contributions, Dr. Guerrant is instrumental in shaping tropical medicine training in the United States.

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