Cryptosporidium parvum, a ubiquitous, highly infectious protozoan parasite, has recently emerged as a major worldwide public health concern. The single most dramatic event that highlighted emerging concerns of the scientific community, public health officials, and drinking water providers was a waterborne outbreak in Milwaukee in 1993 in which 403,000 people became ill, and 54 died. In response to rising concern, cryptosporidiosis was targeted for active surveillance in 1997 under the Foodborne Disease Active Surveillance Network (FoodNet) program as an emerging pathogen. This is a joint initiative in which the Centers for Disease Control and Prevention, the Food and Drug Administration, and the USDA Food Safety and Inspection Service (USDA-FSIS) carry out active surveillance in collaboration with state and local health departments. In 1997, 468 confirmed identifications of Cryptosporidia spp were made at 4 FoodNet survey sites in California, Connecticut, Minnesota, and Oregon. In 1998, the number of survey sites was extended to 7 to include sites in Georgia, Maryland, and New York, and 565 confirmed identifications of Cryptosporidia spp were made.

Since the original identification of C parvum in 1912 in the small intestines of mice, this species has been identified in more than 80 species of mammals worldwide. However, it was not until the 1970s that C parvum was recognized as a causative agent for diarrhea in young calves and other livestock. In 1976, C parvum was recognized as an enteric human pathogen associated with diarrhea in immunocompetent and immunocompromised hosts. In immunocompetent individuals, the acute diarrheal episode is usually self-limiting and lasts 7 to 14 days, along with abdominal pain, vomiting, anorexia, fever, flu-like symptoms, and weight loss. However, in patients with Acquired Immunodeficiency Syndrome (AIDS), the intensity and duration of diarrhea and accompanying malabsorption and dehydration have potentially fatal consequences. Other vulnerable individuals include young children, pregnant women, and individuals being treated with immunosuppressive drugs.

Cryptosporidium is 1 of 5 coccidian genera that infect humans. Molecular techniques allow detection of different gene sequences among isolates of oocysts thought to be C parvum and similarities among oocysts of different species. Cryptosporidium parvum, C muris, C wrairi, and C felis have been identified in mammals, C meleagridis and C baileyi in birds, C serpentis in reptiles, and C nasorum in fish. Cryptosporidium parvum remains the primary focus of public health concern. Evidence is emerging that suggests the existence of several genotypes of C parvum, one with a life cycle confined to humans, independent of the cycle in other mammals. This development is important with regard to epidemiologic investigations that may have a bearing on instituting proper control measures against C parvum. The purpose of this report was to examine the potential role of food as a vehicle in the spread of cryptosporidiosis.

Life Cycle

The life cycle of C parvum, including asexual and sexual components, may be completed within a single host and has unique characteristics. Oocysts released into the environment via feces of infected hosts are fully sporulated and immediately infective. When ingested in contaminated food or water by a new host, the 4 infective sporozoites within each oocyst are released into the small intestine. Sporozoites penetrate the brush border, where they are uniquely located in an intracellular yet extracytoplasmic position. As the functional integrity of the brush border is destroyed, with subsequent loss in viable absorptive surface, profuse diarrhea with severe fluid loss ensues. Progressive, severe fluid loss in AIDS patients with cryptosporidiosis is considered to be a critical factor contributing to death; these patients may typically lose 3 to 6 L of fluid/d, and in extreme instances, as much as 17 L/d. Sporozoites, through an autoinfective process, perpetuate the infective process during asexual proliferation by infecting other cells. Infection is also perpetuated via excystation of 20% of special naked oocysts within the lumen of intestine; these oocysts are produced by sexual reproduction and have extremely thin walls.

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Animal Infection

Because of lack of host specificity, *C. parvum* infects a broad range of mammalian species. It is, however, primarily a disease of young animals. In addition to creating a public health issue of large magnitude, illness in calves and lambs is also a serious economic concern to farmers.

In the United States, 90% of the dairy farms and > 40% of beef operations possess infected cattle. Nearly 50% of 1- to 3-week-old unweaned dairy calves may be infected, whereas prevalence of infection decreases to 22% and 15% for calves that are 3 to 5 weeks old and > 5 weeks old, respectively. In beef operations, 20% of fecal samples submitted from diarrheic calves contained *Cryptosporidium* spp, whereas 11% of samples from nondiarrheic calves contained *Cryptosporidium* spp.

Human Infection

Data from developed countries indicate high seroprevalence; almost a third of a typical population has high antibody titer. On the basis of direct testing for oocysts in feces from patients in 1986, an overall nonoutbreak-associated prevalence of 1 to 2% in Europe and 0.6 to 4.3% in North America was reported. Prevalence of infection in immunocompetent human patients with diarrhea and a control population without diarrhea in industrialized countries was 2.2 and 0.2%, respectively, whereas prevalence in similar populations in developing countries was 6.1 and 1.52%, respectively. In human immunodeficiency virus-infected patients from industrialized countries, patients with diarrhea had a 14% rate of infection, compared with 0% in patients without diarrhea; corresponding rates for developing countries were 24 and 5%, respectively. In Asia, Africa, and Latin America alone, an estimated 200 to 500 million human infections occur annually. Unlike other animals, humans of various ages are commonly infected, although children between 1 and 5 years of age are especially prone to infection.

Environmental Contamination

Widespread shedding of *Cryptosporidium* oocysts in feces of domestic and wild animals (especially young animals) and humans is common. Infected cattle and humans shed millions of environmentally stable oocysts that are immediately infective via fecal-oral contamination. Calves may shed 10⁶ oocysts/d, and humans may shed 10⁵ to 10⁶ oocysts/g of feces. Typically, shedding in humans and other animals may last as long as 2 weeks, but this period may be substantially longer in immunocompromised individuals. Oocysts are especially stable in moist environments such as effluent discharges and agricultural runoffs, which contributes to contamination of surface waters and public water supplies. Contamination of surface waters in the United States ranges from 55 to 87%. The main source of surface water contamination is runoff from agricultural and dairy lands treated with slurry, as well as raw animal waste on farms. Sewage effluents contaminated by fecally contaminated waste discharges and agricultural runoffs have high concentrations (eg, 5,180 oocysts/L) of oocysts. Even treated sewage is substantially contaminated and may contain as many as 1,060 oocysts/L.

Transmission

**Waterborne transmission**—Waterborne transmission is common in humans. Because a large quantity of water is used in processing a variety of food products, contaminated water may be a potential vehicle for transmitting oocysts in the food chain.

The first waterborne cryptosporidiosis outbreak in the United States was reported in Texas in 1984, where a community water supply from an artesian well was contaminated. The 1993 Milwaukee outbreak was caused by inadequacy of the plant filtering process, even though the facility was operating in compliance with existing regulations. Other large outbreaks associated with water supplies occurred in Oregon in 1992 and Georgia in 1987, where 13,000 people became ill.

*Cryptosporidium* oocysts are extremely resistant to disinfectants that are usually effective against other organisms of public health concern in water supplies. Only a few disinfectants in high concentration, such as 10% formaldehyde, 5 to 10% ammonia, and 70% commercial bleach, are capable of destroying oocyst infectivity. Water purification through filtration is also problematic, because the oocysts are small (diameter, 4 to 5 μm). Current standards for water and water treatment facilities are based on bacteriologic criteria such as coliform testing and cannot ensure freedom from *Cryptosporidium* contamination. In 2 recent studies, oocysts were detected in 17% and 27% of drinking water samples from plants operating within existing regulations.

Failure of water treatment purification systems may be caused by a sudden influx of oocysts into the facilities from upstream contamination that in some instances may be triggered by excessive rain. Exposure to the resultant contaminated drinking water may cause large community outbreaks with acute illness if the population has not been exposed previously and has large numbers of susceptible persons, or low-grade endemic transmission. If the population has acquired a degree of protective immunity from prior exposure. However, low concentrations of oocysts in a public water supply do not alone pose a risk to the exposed population.

One of the underlying problems with water testing procedures is lack of specificity in differentiating *C. parvum* from other *Cryptosporidium* species, including those that infect birds and fish, that may not be of public health concern. For example, cross-reactivity may be encountered among *C. meleagris* (turkey), *C. wrairi* (guinea pig), and some isolates of *C. muris* when immunofluorescence methods are used to identify oocysts in water. Algae and suspended solids may also interfere with specificity of some routine assays. Additionally, these tests do not determine whether oocysts are viable. The importance of different strains of *C. parvum*, which may have differences in virulence and infective dose, is also unknown.

Contaminated recreational water is another source of waterborne infection. Control of such contamin-
tion is possible primarily through educating people with diarrhea or recent diarrheal episodes; admonitions not to use swimming pools and other water recreation facilities may be essential to control some epidemics.

Zoonotic transmission—Zoonotic transmission involving young livestock and companion animals is well recognized and occurs most commonly via direct contact with young infected animals. Because of repeated exposure, animal handlers and veterinary students are especially prone to zoonotic infection. Urban residents may have limited contact with animals and, therefore, less low-level exposure to Cryptosporidia organisms, resulting in limited protective immunity. This may create greater risk of acquiring infection when urban residents visit farms and handle animals closely.

Person-to-person transmission—Person-to-person transmission occurs via the fecal-oral route and may be most prevalent in urban settings where there is less contact with animals. Examples include day care centers for children, hospitals, and amongst family members. Risk is increased by the fact that some patients continue to shed infective oocysts for days or weeks after clinical recovery. Transmission from humans to other mammals is also possible.

Foodborne transmission—Results of 1 study indicate that the causative agent is not identified in approximately half of foodborne outbreaks of human disease. Results of another study indicate that only 2% of disease outbreaks associated with food can be attributed to parasitic infections, whereas 79% of disease outbreaks associated with food can be attributed to bacterial infections. Protozoan parasite infections may be underdiagnosed by a factor of 10 or more. This contrasts with the reported number of foodborne outbreaks caused by Escherichia coli O157:H7 and Salmonella spp., for which adequate detection methods exist, even though the infectious doses for these bacteria are quite comparable to that of cryptosporidiosis. Direct evidence to implicate Cryptosporidium contamination in food is difficult, because routine sensitive detection techniques are lacking.

Cryptosporidium parvum does not colonize muscle and edible viscera in living hosts, but when these tissues become contaminated they may serve as vectors for further transmission. Unlike bacteria, Cryptosporidium oocysts cannot multiply in the environment or contaminated food. Nevertheless, by virtue of being resistant to adverse conditions, oocysts can easily be widely disseminated through foods. However, oocyst detection in food is hampered by lack of enrichment and amplification procedures routinely used for identification of bacteria. In addition, unless an outbreak develops, affected individuals may be overlooked, because physicians may not recognize the need for ordering specific diagnostic tests for patients with diarrhea. Humans are highly susceptible to a low infective dose (ID) of cryptosporidial organisms; an ID₅₀ of 134 has been reported, and as few as 30 oocysts may trigger clinical infection in immunocompetent individuals without any prior exposure. Theoretically, even a single oocyst may initiate infection.

The transmission of enteric bacterial pathogens such as E. coli O157:H7, Salmonella spp., and Campylobacter spp to humans via food (as a vehicle) originating from the slaughterhouse environment is well documented. Cryptosporidia organisms could cause similar contamination, enhanced by highly resistant infectious oocysts that are likely to survive routine sanitizing procedures. Although primarily a disease of young livestock, market age swine and cattle without diarrhea are known to shed infectious oocysts as well.

Poultry slaughterhouse effluent may contain Cryptosporidium species that are not of public health concern, although poultry are common carriers of this organism; 80 and 38% of 17-day-old and 24-day-old turkey poults, respectively, from commercial flocks were found to be disseminating oocysts in their feces. Approximately half of the broiler flocks in the Delmarva area were seropositive for Cryptosporidium spp. As many as 137,000 oocysts/L were detected in the raw sewage effluent of 1 cattle slaughterhouse; 149,000 oocysts/L were detected in effluent of another slaughterhouse. However, Cryptosporidium species identification was not reported in these studies. Some of these oocysts may have been C. muris, which is a commensal inhabitant of the abomasum of a small percentage of US cattle and does not have public health importance.

The first well-documented report of foodborne transmission of cryptosporidiosis involved contaminated fresh-purshased cider in Maine in 1993. Some apples that had fallen on the ground were used in preparation of the cider, and it was speculated that they were contaminated by cattle feces. A second outbreak that implicated apple cider occurred in Connecticut and New York; the cider was commercially produced but unpasteurized. Only picked apples had been used, and contamination may have occurred during the washing process through fecal contamination of water. A foodborne outbreak of diarrhea involving approximately 50 people attending a social event occurred in Minnesota; a food handler with poor hygienic practices most likely contaminated chicken salad that was served during the function. The food handler operated a licensed daycare center at the premises and had contact with children, including changing their diapers; inadequate hand washing after each diaper change may have resulted in food contamination when the salad was prepared. In a foodborne outbreak in Spokane, Washington, involving banquet food with 18 separate food and beverage items, results of a case-control study suggested that items containing green onions were the source of infection. It was not clear how the onions became contaminated.

Epidemiologic evidence suggests that foodborne transmission of cryptosporidiosis caused by consumption of contaminated water or irrigated food crops may be responsible for many outbreaks of diarrhea in various countries. Inadequately pasteurized or raw milk and raw sausage have also been implicated.
Genetic Variation

Results of immunologic and molecular genetic studies suggest there are differences in molecular characteristics of Cryptosporidium spp oocysts from human and bovine sources. Strains that adapt as a result of host- or parasite-determined conditions may be transmitted between humans and livestock, including cattle. Moreover, C parvum in humans may be operating in a life cycle independent of the life cycle in animals. On the basis of polymerase chain reaction (PCR) amplification and sequencing analysis of C parvum from bovine and human sources, it was concluded that C parvum may be differentiated into genotype 1 and genotype 2. In humans, C parvum has 2 independent life cycles; genotype 1 is strictly propagated through human-to-human transmission, whereas genotype 2 is transmitted nonspecifically between humans and other animals. Physicians may harbor these 2 genotypes concurrently. Only genotype 2 isolates could be transmitted to mice and calves under routine experimental conditions, which will probably have important implications as an epidemiologic tool in tracing the point source of contamination during an outbreak.

Interestingly, the 1993 Milwaukee cryptosporidiosis outbreak was speculated to be the result of point-source contamination from Lake Michigan, perhaps via overflowing contaminated human sewage, slaughterhouse effluent discharge, or fecal runoff from livestock (especially cattle). However, PCR analysis of 4 isolates from the outbreak revealed that they were genotype 1 and, therefore, of human origin. Although these 4 isolates represented a small proportion of the organisms involved in that large outbreak, their identification does suggest a possible human role in transmission that needs to be studied further.

Polymerase chain reaction-based methodology was instrumental in delineating epidemiologic aspects of at least 2 other outbreaks. In an outbreak of cryptosporidiosis that involved unpasteurized apple cider, results of an epidemiologic study implicated apples on the ground contaminated with calf feces as a probable source; this was corroborated by confirming the C parvum isolate as genotype 2. Similarly, risk factors identified during an outbreak at a day camp suggested a nonzoonotic mode of transmission via an outdoor water faucet contaminated with C parvum. By use of PCR amplification and sequencing methodology, 5 isolates from that outbreak were confirmed to be genotype 1.

Control

Preventive measures are the primary means to control the potential spread of cryptosporidiosis on farms and in slaughterhouses. Instituting general hygienic measures that minimize cross-contamination by keeping the calf rearing area clean may minimize exposure to calves. Introduction of young calves from unidentified sources into a herd should be avoided. Another approach that has shown promise is to provide protection to calves via oral administration of vaccines with inactivated C parvum oocysts; calves given an oral inoculum of lyophilized C parvum oocysts had significant protection, compared with control calves. Humans, especially those within susceptible populations, should thoroughly wash their hands after contact with farm animals and pets.

The Hazard Analysis and Critical Control Points (HACCP) model of meat and poultry inspection currently being implemented by the USDA-FSIS to improve food safety provides a framework of preventive actions to eliminate or reduce the introduction of pathogens into the food supply. A major emphasis is to control contamination with enteric pathogens by eliminating fecal contamination by implementing a policy of zero tolerance. Presently, most attention is being directed at controlling E coli O157:H7, Salmonella spp, and Listeria spp. However, because this is not a pathogen-specific control program, it is meant to control transmission of many pathogens, including Cryptosporidium spp. In 1998, the incidence of laboratory-confirmed Cryptosporidium identifications at 4 sites was 7% lower than reported in FoodNet surveys of the same sites in 1997. Although this decrease could be attributed to annual fluctuations, it does suggest that the HACCP program implemented in large meat and poultry processing plants since January 1998 may have had some effect. However, the contribution of slaughterhouses to the transmission of Cryptosporidiosis spp, relative to other routes of transmission, is not fully understood at this time.

Heating for 30 minutes at 65°C destroys Cryptosporidium oocysts. Moist heat treatment is more effective, because oocysts in calf feces and intestinal contents are rendered noninfectious when heated in moist conditions from 9 to 35°C for 15 to 20 minutes or heated at 45°C for 5 to 20 minutes. Results of another study indicate that when water containing oocysts reaches 64.2 or 72.4°C, infectivity is lost after 2 minutes or 1 minute, respectively. Commercial pasteurization with high temperature for a short period (71.7°C for 15 seconds) is effective in destroying infectivity of Cryptosporidium oocysts in milk and water. Thus, 2 intervention strategies, steam pasteurization and high-temperature steam vacuuming, that have been recently used in slaughter operations may be effective in decreasing contamination, although these methods have not been tested specifically for oocyst inactivation.

In immunocompetent humans, infection is generally self-limiting and is kept in check as acquired immunity increases. However, preventive measures to safeguard immunocompromised individuals are much more stringent, because, theoretically, ingestion of even 1 oocyst could initiate a severe infection. It is prudent for immunocompromised individuals to boil drinking water for 1 minute to kill all infective agents or use a filter system that effectively removes Cryptosporidium oocysts (eg, a 1-µm filter system). Various freezing, drying, freeze-drying, and heating food-preservation methods are known to inactivate oocysts in food to varying degrees. Although there is no clear understanding of the effect of various processing techniques used in the manufacture of meat byproducts on the survivability of Cryptosporidium oocysts, it is generally believed that processed products do not pose a risk to humans.
References


