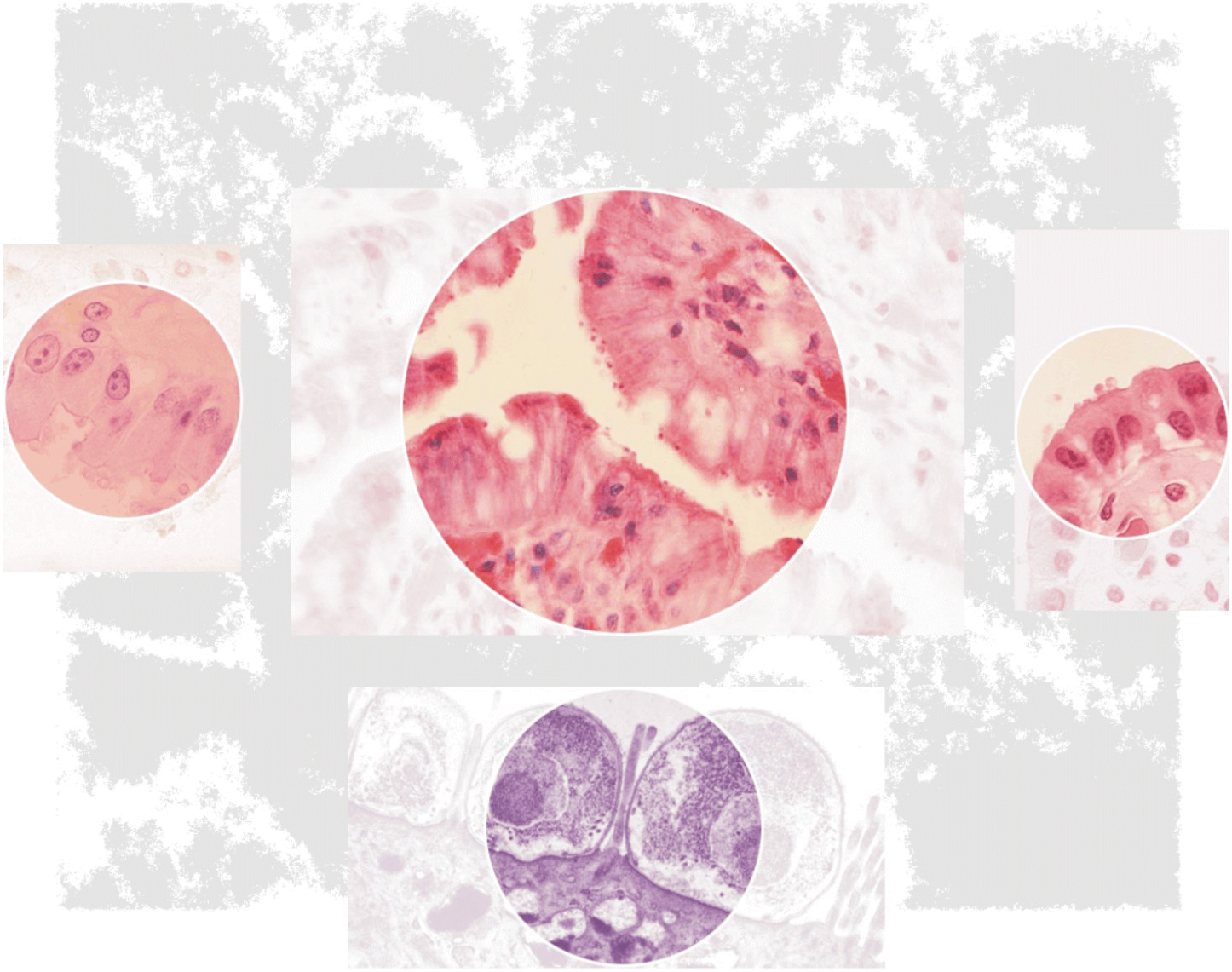
United States
Environmental Protection
Agency

Office of Science and Technology
Office of Water
Washington, DC 20460

EPA-822-R-01-009
March 2001
www.epa.gov

Cryptosporidium: Drinking Water Health Advisory



I. Introduction

Purpose

The Health Advisory Program, sponsored by the Office of Water (OW), provides information on the health effects, analytical methodology, and treatment technology that would be useful in dealing with the contamination of drinking water. Most of the Health Advisories prepared by OW are for chemical substances. This Health Advisory is different in that it addresses contamination of drinking water by a microbial pathogen, including the issues of infective dose (i.e., the number of particles of a pathogen necessary to cause an infection in a host) and pathogen control. Therefore, the format and contents of this Health Advisory necessarily vary somewhat from the standard Health Advisory document.

Health Advisories serve as informal technical guidance to assist federal, state, and local officials responsible for protecting public health when emergency spills or contamination situations occur. They are not to be construed as legally enforceable federal standards. The Health Advisories are subject to change as new information becomes available.

This Health Advisory summarizes the information presented in the Office of Water's Criteria Document for *Cryptosporidium* (USEPA, 1994) and its addendum (USEPA, 2001b). Individuals desiring further detail should consult these documents, which are available from the U.S. Environmental Protection Agency, OW Resource Center, Room M6099; Mail Code: PC-4100, 401 M Street, S.W., Washington, D.C. 20460; the telephone number is (202) 260-7786. The documents also can be obtained by calling the Safe Drinking Water Hotline at 1-800-426-4791.

Summary

Cryptosporidium oocysts are common and widespread in ambient water and can persist for months in this environment. The dose that can infect humans is low, and a number of waterborne disease outbreaks caused by this protozoan have occurred in the United States, most notably in Milwaukee, Wisconsin, where an estimated 400,000 people became ill in 1993. Otherwise healthy people recover within several weeks after becoming ill, but illness may persist and contribute to death in those whose immune systems have been seriously weakened (e.g., AIDS patients). Drugs effective in preventing or controlling this disease are not yet available. The public health concern is worsened by the resistance of *Cryptosporidium* to commonly used water disinfection practices such as chlorination. However, a well-operated water filtration system is capable of removing at least 99 of 100 *Cryptosporidium* oocysts in the water. Monitoring for this organism in water is currently difficult and expensive. EPA believes that there is sufficient information to conclude that *Cryptosporidium* may cause a health problem and occurs in public water supplies at levels that may pose a risk to human health.

II. General Information

History

- *Cryptosporidium* was described by Tyzzer in 1907 but remained medically unimportant to humans until the first cases of cryptosporidiosis in humans were reported in 1976 by Nime *et al.* and Miesel *et al.* (Fayer *et al.*, 1997a). *Cryptosporidium* was first recognized as a waterborne pathogen during an outbreak in Braun Station, Texas (1984), in which more than 2,000 individuals were afflicted with cryptosporidiosis (D'Antonio *et al.,* 1985; Graczyk *et al.,* 1998a). Since that time, outbreaks affecting more than one million individuals have been documented

throughout North America and Europe, with the single largest epidemic occurring in Milwaukee, Wisconsin, in 1993 (Mackenzie *et al.,* 1994).

Organism Description

Taxonomy

- *Cryptosporidium* is one of several protozoan genera in the phylum Apicomplexa, which develop within the gastrointestinal tract of vertebrates throughout their entire life cycles (Fayer *et al.*, 2000). Apicomplexans are obligate intracellular parasites. They are characterized by the presence of special organelles located at the tips (apexes) of cells that contain materials used to penetrate the host cells and establish successful infections. Examples of Apicomplexa other than *Cryptosporidium* include *Plasmodium* (the causative agent of malaria) (Tortora *et al.*, 1994).

- The taxonomy of the genus *Cryptosporidium* is uncertain and changing. The current classification scheme entails ten species of *Cryptosporidium* (Fayer *et al.*, 2000). Table 1 lists these ten *Cryptosporidium* species and the host organism(s) in which each parasite was originally found; some of these species have since been shown to occur in additional hosts (Fayer *et al.*, 2000; Fayer *et al.*, 1997a). *Cryptosporidium* has been observed in over 150 mammalian species (Fayer *et al.*, 2000); however, illness in humans is confined primarily to infections associated with *C. parvum* (O'Donoghue, 1995).

Table 1. Valid *Cryptosporidium* Species

***Cryptosporidium* Species**	**Initially Described Host Species**
C. andersoni	*Bos taurus* (cattle)
C. baileyi	*Gallus gallus* (domestic chicken)
C. felis	*Felis catis* (domestic cat)
C. meleagridis	*Meleagris gallopavo* (turkey)
C. muris	*Mus musculus* (house mouse)
C. nasorum	*Naso literatus* (fish)
C. parvum	*Mus musculus* (house mouse)
C. saurophilum	*Eumeces schneideri* (skink)
C. serpentis	*Elaphe guttata* (corn snake) *E. subocularis* (rat snake) *Sanzinia madagasarensus* (Madagascar boa)
C. wrairi	*Cavia porcellus* (guinea pig)

Source: Adapted from Fayer *et al.* (2000) and Fayer *et al.* (1997a)

- The taxonomy of *Cryptosporidium* is in the forefront of current research on the parasite, and changes may be forthcoming. Molecular studies have found considerable evidence of genetic

heterogeneity among isolates of *C. parvum* from different vertebrate species, and findings from these studies indicate that a series of host-adapted genotypes or strains of the parasite exist (Awad-El-Kariem *et al.*, 1998; Fayer *et al.*, 2000; Morgan *et al.*, 1999a: Morgan *et al.*, 1999b; Morgan *et al.*, 1999c; Morgan *et al.*, 1999d; Morgan *et al.*, 1998; Spano *et al.*, 1998a; Spano *et al.*, 1998b; Sulaiman *et al.*, 1998; Xiao *et al.*, 1999a; Xiao *et al.*, 1999b).

- Separate subpopulations within the *C. parvum* strain exist, one that infects primarily humans and one that infects primarily animals (Carraway *et al.,* 1994; Awad-El-Kariem, 1996; Awad-El-Kariem *et al.*, 1998). Two genotypes with genetic differences among adhesion proteins have been found; the H (human) genotype was found exclusively in human isolates and the C (cattle) genotype was found in both calf isolates and in isolates from human patients reporting exposure to infected cattle (Peng *et al.*, 1997).

- In addition to the human and cattle genotypes, characterizations of *C. parvum* isolates from other vertebrate species have revealed host-specific genotypes in mice, pigs, marsupials, and dogs (Fayer *et al.*, 2000; Morgan *et al.*, 2000; Morgan *et al.*, 1999a; Morgan *et al.*, 1999b; Morgan *et al.*, 1999c; Morgan *et al.*, 1999e; Morgan *et al.*, 1998; Pereira *et al.*, 1998; Xiao *et al.*, 1999b).

Life Cycle/Morphological Features

- *Cryptosporidium* has a complex life cycle, which is completed in one to eight days and takes place within the body of the host (either humans or any of a wide variety of animal species). *Cryptosporidium* is excreted in the feces of an infected host in the form of an oocyst. The oocyst represents the only stage of the life cycle that exists outside the host and consists of four sporozoites housed within a sturdy, multi-layered wall. Oocysts of *C. parvum* are small, generally measuring four to six micrometers in diameter and are spherical-to-ovoid in shape (Fayer and Ungar, 1986). The life cycle is repeated when sporulated oocysts excreted by an infected host are ingested by a new host and the sporozoites excyst within the small intestine. A complete description of the life cycle of *Cryptosporidium* is provided in the 1994 USEPA *Cryptosporidium* Criteria Document (see Figure II-1, p. II-5).

- Robertson *et al.* (1993, 1994) provided evidence that the suture spanning part of the circumference of the oocyst inner wall described in ultrastructural studies is not the same structure as the apparent "fold" in the oocyst wall seen using fluorescence microscopy. Their ability to reversibly induce the folds suggests that this structure is probably artifactual. As a result, the researchers recommend that the apparent fold no longer be considered a diagnostic feature of *Cryptosporidium parvum.*

Environmental Fate

- The thick-walled oocyst is appreciably resistant to natural decay in the environment as well as to most disinfection processes. Walker *et al.* (1998) reviewed laboratory and field studies on the survival and transport of *C. parvum* oocysts. Oocysts can survive for months in soil under cool, dark conditions and for up to one year in low-turbidity water. Infectivity appears to be lost when oocysts are frozen, freeze-dried, boiled, or heated at or above 60°C for 5 to 10 minutes (Badenoch *et al.*, 1990).

- In general, shorter freezing times are required to neutralize infectivity when lower freezing temperatures are used (e.g., 1 hour at -70°C vs. 168 hours at -15°C to completely neutralize infectivity) (Fayer and Nerad, 1996). Robertson *et al.* (1992) demonstrated that oocysts were inactivated after incubation at -22°C for 18 days.

- Water temperature can affect oocyst infectivity; Fayer *et al.* (1998) demonstrated that oocysts retained their infectivity for 1 week in -10°C water but remained infectious for up to 24 weeks in 20°C water. Warming oocysts to 45°C for 5 to 20 minutes was effective in completely neutralizing their infectivity (Anderson, 1985). Under conditions of high water temperatures, Fayer (1994) indicated that all evidence of *C. parvum* infectivity was lost within 60 seconds when temperatures exceeded 72°C or when temperatures of at least 64°C were maintained for 2 minutes. Harp *et al.* (1996) demonstrated that oocysts suspended in water or milk lost infectivity after heating to 71.7°C for 5 to 15 seconds in a laboratory-scale pasteurizer.

- The infectivity of oocysts from calf fecal samples which had been subjected to drying in either the summer (i.e., 18°C to 29°C, 60% humidity) or winter (i.e., -1°C to 10°C, 60% humidity) was completely lost within 1 to 4 days (Anderson, 1986).

- *C. parvum* oocysts are more resistant to chemical agents than the majority of protozoa. A complete description of the morphological features of each life cycle stage of *Cryptosporidium* (oocyst, sporozoite, trophozoite, merozoite, microgametocyte, macrogametocyte) is provided in the 1994 *Cryptosporidium* Criteria Document (see pp. II-7 – II-9 of the 1994 document).

Species Transmission

- *Cryptosporidium* can be transmitted from person to person, or from farm livestock (e.g., cattle, sheep, or pigs) to humans, through the fecal-oral route (Casemore, 1990). Ingestion of drinking water contaminated with oocysts is the major mode of transmission. Other routes of transmission include fecal contamination of fomites (i.e., inanimate objects such as clothes, pens, doorknobs) and contamination of recreational waters (e.g., swimming pools).

Direct Transmission Between Humans

- A number of studies have shown that person-to-person transmission of cryptosporidiosis infection may occur within family homes, day-care centers, hospitals, and in urban environments where population densities are high (USEPA, 1994). The route of infection is either direct, through fecal-oral contact, or indirect, through fomites. The rate of transmission between immunocompromised individuals is higher than between immunocompetent individuals (Heald and Bartlett, 1994). Secondary transmission of cryptosporidiosis has also been observed among humans whose occupation places them near primary cases within a confined space. For example, an outbreak occurred among crew members on a U.S. Coast Guard cutter that had obtained water from the city of Milwaukee during the 1993 epidemic (Moss *et al.,* 1994). It was suggested that the disease was transmitted from person to person. It is difficult, however, to distinguish between primary infections (i.e., those due to ingestion of contaminated water) and secondary infections (i.e., those due to contact with fecal contaminated fomites, food, or other infected individuals). In some instances it may not be possible to determine whether transmission between humans is

the primary cause of cryptosporidiosis, especially in situations when humans have also come into contact with animals through occupational or recreational activities (Adam *et al.*, 1994).

- Infected individuals will shed oocysts in their feces and can be expected to transmit the infection to other family or community members. In addition, day-care centers are a potential source for secondary spread of cryptosporidiosis because of their high density of a susceptible population and the inadequate personal hygiene habits of the children.

Transmission from Animals to Humans

- The 1994 USEPA *Cryptosporidium* Criteria Document cites adequate evidence for the transmission of *Cryptosporidium* from animals, particularly livestock, to humans. Of ten *Cryptosporidium* species infecting vertebrates, only one, *C. parvum*, represents a global public health problem due to its zoonotic potential (Graczyk *et al.*, 1998a).

- Transport of oocysts through migratory waterfowl may have epidemiological implications, as the birds can consume and transport *C. parvum* even though they are not susceptible to infection. In experimental studies, *C. parvum* oocysts retained their infectivity after being excreted in the feces of ducks and/or geese dosed orally (Fayer *et al.*, 1997b) or by intubation (Graczyk *et al.*, 1996; Graczyk *et al.*, 1997). In another study, *C. parvum* oocysts that were recovered from goose fecal samples collected in the Chesapeake Bay successfully infected laboratory mice (Graczyk *et al.*, 1998b). Viable *Cryptosporidium* oocysts have been found in fecal samples and cloacal lavages of gulls which fed sewage or other refuse (Smith *et al.*, 1993). Transmission from waterfowl is most likely to occur around reservoirs or in waters where shellfish are harvested for human consumption.

- Insects have also been shown to carry *C. parvum* oocysts on their outer surfaces as well as in their intestinal tracts. House flies (*Musca domestica*) exposed to bovine feces containing *C. parvum* oocysts transported oocysts to other surfaces via fecal deposition (Graczyk *et al.*, 1999). This study also demonstrated that oocysts were found on the exoskeletons and in the intestinal tracts of the exposed flies. In a study by Mathison and Ditrich (1999), oocysts were collected on the external surfaces and in the intestinal tracts of dung beetles exposed to *C. parvum* oocyst-supplemented dung. Zerpa and Huicho (1994) reported a case of cryptosporidial diarrhea in a 20-month-old male child in which *Cryptosporidium* oocysts were detected in the digestive tract of cockroaches (*Periplaneta americana*) found in the garden of the child's home. No other potential sources of infection were identified.

III. Occurrence

Worldwide Distribution

- *Cryptosporidium* occur in numerous mammalian, avian, reptilian, piscine, and amphibian hosts worldwide (Fayer, 1997; Fayer *et al.*, 2000; Hoover *et al.*, 1981; O'Donoghue, 1995).

- Since 1982, human cryptosporidiosis has been documented in 95 countries on every continent except Antarctica (Fayer, 1997). Human cryptosporidiosis occurs in developed and developing

countries, urban and rural areas, and in temperate as well as tropical climates (Fayer, 1997; O'Donoghue, 1995).

Surface Waters

- *Cryptosporidium* may be more common in surface water than ground water because surface waters are more vulnerable to direct contamination from sewage discharges and runoff. Lisle and Rose (1995) reported that between 5.6% and 87.1% of source waters (i.e., surface, spring, and groundwater samples not impacted by domestic and/or agricultural waste) tested contained 0.003 to 4.74 *Cryptosporidium* oocysts/L. In another major study, LeChevallier and Norton (1995) reported finding oocysts in 60.2% of surface waters tested in the U.S. and Canada. However, all surface waters are subject to a complex set of watershed processes and characteristics that may lead to the presence of *Cryptosporidium* oocysts (Crockett and Haas, 1997; States *et al.*, 1997; LeChevallier *et al.*, 1997).

- *Cryptosporidium* oocysts have also been found in more than 50% of raw sewage samples (Bukhari *et al.*, 1997; Zuckerman *et al.*, 1997), 4.5% of raw water samples, and 3.5% of treated water samples (Wallis *et al.*, 1996). Ong *et al.* (1996a, 1996b) found that water from rivers flowing through cattle pastures in British Columbia exhibited higher *Cryptosporidium* counts than did water in a protected watershed.

Ground Water

- *Cryptosporidium* oocysts are found less frequently in ground water than in surface water, although new data contradict previous assumptions that ground water is inherently free of parasites such as *Cryptosporidium.* For example, Hancock *et al.* (1998) recently reported a study of 199 ground water samples tested for *Cryptosporidium.* They found that 5% of vertical wells, 20% of springs, 50% of infiltration galleries, and 45% of horizontal wells tested contained *Cryptosporidium* oocysts.

Soil

- Limited studies have been performed to ascertain the presence or viability of *Cryptosporidium* in soil in several documented outbreaks. However, transport of *Cryptosporidium* oocysts to water from feces-contaminated soil during weather events has been suggested as the most probable mechanism of source water contamination (Kramer *et al.,* 1996). Vertical movement of oocysts can occur through the soil, as demonstrated in 30-cm soil cores (Mawdsley *et al.*, 1996a). Twenty-one days after inoculation, the majority of oocysts still in the soil remained in the top two cm of the soil cores, but some were found as deep as 30 cm. The number of oocysts recovered decreased with increasing soil depth. Another study by Mawdsley *et al.* (1996b) confirmed these results but also suggested that a large proportion of oocysts are retained in the runoff rather than being adsorbed onto the soil surface.

Foods and Beverages

- Several foodborne outbreaks in recent years have highlighted the role of *Cryptosporidium* as a foodborne pathogen. The presence of *Cryptosporidium* has been documented in raw milk

(Badenoch *et al.*, 1990), unpasteurized apple cider (Millard *et al.*, 1994), uncooked meat products (Casemore *et al.*, 1997), uncooked (and possibly unwashed) green onions (Quinn *et al.*, 1998), and fresh produce (Monge and Chinchilla, 1995). Refrigeration does not affect oocyst viability (Friedman *et al.*, 1997).

Environmental Factors Affecting *Cryptosporidium* Survival

- In the absence of freezing conditions, colder water temperatures tend to promote the survival of most microorganisms. For example, *C. parvum* oocysts may survive outside of mammalian hosts for several months or more depending upon water temperature (Straub *et al.*, 1994). In freezing conditions, *C. parvum* oocysts are not necessarily rendered noninfectious (Fayer *et al.*, 1991; Fayer, 1997). Oocyst stability under freezing conditions is at least partially dependent upon the surrounding matrix. For example, fecal material can confer a cryopreservative effect (Satter *et al.*, 1999).

- Under conditions of high water temperatures, Fayer (1994) indicated that all evidence of *C. parvum* infectivity was lost within 60 seconds when temperatures exceeded 72°C or when temperatures of at least 64°C were maintained for 2 minutes. It is important to note, however, that such water temperatures are not typical environmental conditions.

- Physical shear forces may also affect oocyst viability. Such shear forces could result from the potentially abrasive effects of sand and gravel particles or fast-flowing waters. In addition, oocysts could be subject to such shear forces in rapid sand filters. Parker and Smith (1993) demonstrated rapid inactivation of oocysts in a mixed sand reactor.

- Microbial predation may be an important influence on oocyst survival in natural waters. For example, Sattar *et al.* (1999) observed that oocysts incubated in dialysis cassettes suspended in natural waters exhibited significantly longer survival times when bacterial populations were excluded from the suspension water.

Specific Disease Outbreaks

Outbreaks Associated with Drinking Water

- A number of cryptosporidiosis outbreaks have been associated with drinking water (Rose *et al.,* 1997; Solo-Gabriele and Meumeister, 1996). Deficiencies in water treatment systems are often cited as a major reason for outbreaks, and even the best of systems can be overwhelmed by a high density of oocysts entering the source waters over a short period of time. For example, a national survey over a 2-year test period (1993 and 1994) identified 5 outbreaks; these 5 outbreaks resulted in 403,271 cases (Kramer *et al.*, 1996). Of this total, 403,000 were from the outbreak in Milwaukee, Wisconsin, 103 were from Las Vegas, Nevada, and 27 were from an outbreak at a resort in Minnesota. Some notable outbreaks in the United States associated with drinking water are summarized in Table 2 below.

Table 2. Notable Outbreaks of Cryptosporidiosis Associated with Drinking Water in the U.S.

Year	State	Number of Cases	Source	Deficiency
1984	Texas	2006	Ground water	Sewage contamination
1986	New Mexico	78	Surface water	Untreated
1987	Georgia	12,960	River	Treatment deficiency
1991	Pennsylvania	551	Ground water	Treatment deficiency
1992	Oregon	15,000	Spring/river	Treatment deficiency
1993	Wisconsin	403,000	Lake	Treatment deficiency
1993	Washington	7	Private Well	Surface contamination
1993	Minnesota	27	Lake	Unknown
1993	Nevada	103	Lake	Inadequate filtration
1994	Washington	104	Community Well	Sewage contamination
1995	Florida	72	Not applicable	Cross connection

Source: USEPA (2001b)

Outbreaks Associated with Recreational Waters

- Fourteen outbreaks of gastroenteritis related to recreational waters were reported by nine states during 1993 and 1994 (Kramer *et al.,* 1996). Ten of these outbreaks were caused by *Cryptosporidium* or *Giardia*, with five outbreaks specifically linked to *Cryptosporidium*. Three of the *Cryptosporidium* outbreaks were associated with motel swimming pools, and two were associated with community swimming pools. All five pools were filtered or chlorinated. One had a malfunctioning filter, but none of the other pools had identifiable treatment deficiencies. The inability of chlorine at levels normally used in swimming pools to kill *Cryptosporidium,* coupled with inadequate maintenance of pool filtration equipment, has been suggested as the primary cause of swimming pool related cryptosporidiosis. Kramer *et al.* (1998) reported on an outbreak involving 38 individuals who contracted cryptosporidiosis while swimming in a recreational lake. The authors speculated that contamination of the lake came from either infected swimmers or contaminated run-off.

Foodborne Outbreaks

- Foodborne outbreaks of cryptosporidiosis have only rarely been reported. Harp *et al.* (1996) reported that standard commercial pasteurization techniques kill 100% of *C. parvum* oocysts. In October 1993, an outbreak of cryptosporidiosis occurred among students and staff who consumed contaminated apple cider while attending an agricultural fair in central Maine. This incident was the first large outbreak in which foodborne *Cryptosporidium* could be identified and documented as the causative agent (Millard *et al.*, 1994). *Cryptosporidium* oocysts were detected in the stools of 50 (89%) of the primary and secondary case subjects tested. Oocysts were detected in the apple cider, on the cider press, and in the stool specimen of a calf on the farm of the supplier of the apples used to make the cider. This outbreak underscores the need for precautions by agricultural producers to avoid contamination of foodstuffs by infectious agents commonly present in the farm environment.

- Two more foodborne outbreaks, one involving apple cider and another associated with green onions, were reported in a review by Rose and Slifko (1999). A community outbreak in New York was associated with a cider mill using apples picked from an orchard located near livestock. Another outbreak was traced back to a dinner banquet in Washington in which unwashed green onions were the suspected cause (Quinn *et al.*, 1998; Rose and Slifko, 1999).

- The Minnesota Department of Health reported on cryptosporidiosis in 50 attendees of a social gathering who ate a salad contaminated during preparation by a day-care worker (CDC, 1996b).

Outbreaks Among Travelers

- Cryptosporidiosis has emerged as an important cause of traveler's diarrhea, particularly among people visiting developing countries. Travelers to developed countries such as the U.S. have also acquired *Cryptosporidium* infections. For example, MacKenzie *et al.* (1995) reported that visitors to Milwaukee during the 1993 outbreak transmitted the parasite to members of their households upon returning home.

Outbreaks at Day-care Centers

- Several outbreaks of cryptosporidiosis have occurred in day-care centers in the United States; these outbreaks are summarized in "*Cryptosporidium*: Risk for Infants and Children" (USEPA, 2001a).

IV. Health Effects

Animals

Symptoms

- Most cases of cryptosporidiosis in mammals involve infections by *C. parvum* (Fayer, 1997; O'Donoghue, 1995). The most common features of cryptosporidiosis in mammals are profuse diarrhea, dehydration, fever, anorexia, and weight loss.

- In general, the severity of the infection depends on the species, age, and immune status of the host (Fayer, 1997). Infections are primarily seen in younger animals and animals with compromised immune systems, while infected healthy adult animals may be asymptomatic or develop only mild clinical signs (Fayer, 1997; O'Donoghue, 1995).

- Adult animals often appear asymptomatic while shedding small numbers of oocysts (Casemore *et al.,* 1997; Fayer, 1997; O'Donoghue, 1995).

Therapy and Prevention

- Management of cryptosporidiosis in all animals involves a combination of antidiarrheal drugs and anticryptosporidial drugs, along with other preventive measures (e.g., rehydration with fluids and electrolytes) (Blagburn and Soave, 1997).

- Prevention of cryptosporidiosis in domestic animals is best achieved by eliminating contact with viable oocysts as much as possible. This involves isolation of infected animals and disinfection of all articles that come into contact with the infected animals. This is particularly difficult in settings with large numbers of animals such as farms or zoos (Blagburn and Soave, 1997).

- Sources of infection in animals include: other infected animals of the same or different species (e.g., it is believed that rodents can infect calves or cattle with *C. parvum*); mechanical carriers such as insects,

birds and humans; contaminated feed and water; and other contaminated fomites such as bedding, brushes, shovels, and feed utensils (Fayer, 1997).

Humans

Symptoms and Clinical Features

- The clinical manifestations of cryptosporidiosis in humans are directly related to the immunocompetence of the host, and may include profuse, non-bloody, watery diarrhea that usually resolves spontaneously within approximately 48 hours. Variability in clinical symptoms is appreciable and may include renal failure and liver disease (Griffiths, 1998). Other symptoms reported by individuals afflicted with cryptosporidiosis include abdominal cramps, vomiting, lethargy and general malaise.

- The incubation period in humans is estimated to vary between two to ten days (Arrowood, 1997), with a mean incubation of approximately seven to nine days (Juranek and MacKenzie, 1998).

Epidemiological Data

- Because *C. parvum* is ubiquitous, infects most mammals, and is highly infectious, all human populations are at risk of infection to some degree (Griffiths, 1998). Since 1982, human cryptosporidiosis has been reported in almost 100 countries (Ungar, 1990); the impact is greatest in developing countries.

- *Cryptosporidium* is the third or fourth most commonly identified pathogen in the world, and the reported infection rates are higher in developing countries, especially in children. Seasonal and temporal trends vary from country to country and occurrence may indirectly reflect rainfall and farming events such as lambing (Casemore, 1990).

- The occurrence of *Cryptosporidium* infection in Gambian children has seasonal peaks associated with rain and high relative humidity (Adegbola *et al.,* 1994). Factors accounting for the seasonal distribution of *Cryptosporidium*, particularly in developing countries, may include increased survival of oocysts in a high relative humidity environment and an increased possibility of dissemination of oocysts to children as a result of decreased domestic and environmental hygiene in the rainy season.

- Domestic animals such as calves and lambs are common zoonotic reservoirs implicated in occupational exposure, indirect zoonotic transmission, and contamination of food (e.g., sausages, offal, and raw milk). Animals also contribute to environmental contamination in sources such as watersheds, food crops, and recreational waters.

- Cryptosporidiosis may also be associated with nosocomial (hospital-acquired) infections, sexual transmission, or traveler's diarrhea (Casemore, 1990). *Cryptosporidium* is a primary cause of traveler's diarrhea, typically being transmitted through contaminated food or water. Casemore (1990) observed that the severity of disease from infection is greatest among children less than five years of age and among immunocompromised patients.

- Epidemiological data indicates that immunocompromised populations are at high risk of infection with *Cryptosporidium* oocysts. This increased risk has been demonstrated in patients undergoing

chemotherapy for cancer (Tanyuksel *et al.*, 1995), patients with AIDS (Clayton *et al.,* 1994), infants and children (USEPA, 2001a), and the elderly (Logar *et al.,* 1996).

- Cryptosporidiosis is recognized as a significant disease in child care settings (Cordell and Addiss, 1994). The 1994 *Cryptosporidium* Criteria Document discussed the high prevalence of cryptosporidiosis in children and noted that the evidence comes primarily from reports of diarrhea in day-care centers. Furthermore, there have been several reports documenting high prevalences of *Cryptosporidium* in day-care settings (Addiss *et al.*, 1991). Additionally, an outbreak was reported in a day camp where 74% of the 104 persons attending the camp, including 72 of the 98 children and 5 of the 6 counselors, showed symptoms of *Cryptosporidium* infection (CDC, 1996a). Additional information regarding cryptosporidiosis in children is provided in "*Cryptosporidium*: Risk for Infants and Children" (USEPA, 2001a).

Therapeutic Management

- Cryptosporidiosis is self-limiting in most patients (Griffiths, 1998). The recommended management of *Cryptosporidium*-infected immunocompromised patients includes careful monitoring of hydration and electrolyte balance, with oral or intravenous hydration and supplemental nutrition as necessary. Antimotility agents (e.g., opiates or somatostatin and its analogues) may also be helpful in preventing dehydration. Patients co-infected with HIV should continue or begin antiretroviral therapy to suppress viral replication and boost $CD4^+$ cell counts. Patients currently undergoing chemotherapy or immunosuppressive therapy should be removed from treatment.

- The most promising development in the treatment of cryptosporidiosis is associated with the introduction in 1996 of protease inhibitors for the treatment of HIV infection. A decrease in the prevalence of intestinal cryptosporidiosis coincided with the widespread use of protease inhibitors in HIV-infected patients (Le Moing *et al.*, 1998).

- The results of other studies suggest that combination antiretroviral therapy that incorporates a protease inhibitor provides HIV-infected patients the best chance for changing the course of cryptosporidiosis (Maggi *et al.*, 2000; Miao *et al.*, 1999).

- To date, no chemotherapeutic agents have been consistently effective in the management of cryptosporidial infections (Blagburn and Soave, 1997; O'Donoghue, 1995). Although anecdotal success has been reported following treatment with some compounds, most have proven ineffective in controlled studies. As many as 100 compounds have been shown to be ineffective for the treatment of cryptosporidiosis; some of the many compounds that have been investigated including spiramycin, azithromycin, clarithromycin, roxithromycin, diclazuril, letrazuril, paromomycin, nitazoxanide, difluoromethylornithine, and atovaquone (Blagburn and Soave, 1997).

Immunity

- The importance of cellular immunity in resolving *Cryptosporidium* infection is highlighted by the contrasting ability of immunocompetent and immunocompromised individuals to resolve infections (Griffiths, 1998).

- Specific IgG, IgM, IgA, and IgE antibodies have been detected in patients with confirmed *Cryptosporidium* infection (Ungar *et al.*, 1986; Casemore, 1987; Laxer *et al.*, 1990; Kassa *et al.*, 1991); however, the role of these antibodies in combating infection remains unclear (O'Donoghue, 1995).

- There is also evidence in humans for protective immunity to cryptosporidial infection (Reese *et al.*, 1982; Current, 1994; Okhuysen *et al.,* 1998). For example, repeat infections in dairy cattle workers occur but are generally much milder than the first infection (Reese *et al.*, 1982). Furthermore, permanent residents in areas where cryptosporidiosis is common often acquire mild or asymptomatic infections while visitors may become very ill (Current, 1994). Okhuysen *et al.* (1998) reported on the rechallenge of human volunteers previously infected with *Cryptosporidium.* Nineteen healthy, immunocompetent adults were challenged with 500 oocysts one year after a primary infection. Fewer study subjects shed oocysts after the second exposure, compared to their first exposure (16% vs. 63%). Although the percentage of subjects with diarrhea was similar, the clinical severity, as determined by the number of unformed stools passed, was less following rechallenge compared to the primary challenge response. Antibody responses (IgG and IgA) did not correlate to the presence or absence of infection.

Chronic Conditions

- Chronic illness resulting from cryptosporidial infection may manifest itself as a series of intermittent episodes or may be persistent. Duration of illness in cryptosporidiosis is influenced primarily by the immune status of the individual, with most immunocompetent individuals overcoming the acute enteritis stage within two weeks. Chronic enteritis in immunocompromised individuals may last as long as the immune impairment.

- Immunocompromised populations include AIDS patients, patients undergoing chemotherapy for treatment of neoplasms, persons undergoing immune suppression treatment to prevent rejection of skin or organ transplants, malnourished individuals, patients with concurrent infectious diseases such as measles, the elderly. A functional threshold has been established using the number of $CD4^+$ lymphocytes (a specific type of immune cell) to define the probability that infection will resolve; patients with $CD4^+$ counts below 200 cells/ L are most likely to suffer chronic infection (Fayer *et al.*, 1997a).

Mechanisms of Pathogenesis

- Only recently have alternative mechanisms of *Cryptosporidium* pathogenesis been proposed. *Cryptosporidium* sporozoites and merozoites invade the absorptive cells covering the small intestinal villi, damaging and eventually killing the enterocytes. When killed enterocytes are extruded from the intestinal epithelium, crypt cells are signaled to repair the damage. Additionally, there is infiltration of prostaglandin (PGE) secreting inflammatory cells. Both crypt cells and PGE are known to stimulate chloride ion secretion; in addition, PGE inhibits sodium chloride absorption (Clark and Sears, 1996). This disruption in the absorption/secretion balance can lead to diarrhea (Argenzio *et al.*, 1993). Alternatively, it has been suggested that the diarrhea may be caused by a toxin (Guarino *et al.*, 1994; Guarino *et al.,* 1995).

V. Risk Assessment

- Environmental risk assessments based upon exposure to chemical pollutants have historically relied upon a conceptual framework generally considered inadequate for microbial pathogen risk assessment. Although most human populations are assumed to be at risk for cryptosporidiosis to at least some degree, it has been difficult to collect accurate figures describing the prevalence of infection in humans. This is due to limitations in public health reporting systems and to incomplete characterization of oocyst speciation and survival under various environmental conditions. Dose-response data obtained from human volunteer challenge studies contribute to the ability to quantify the risks associated with *Cryptosporidium* exposure.

- The framework for assessing chemical exposures does not account for a number of microbial considerations including: pathogen-host interactions, secondary spread of microorganisms, short- and long-term immunity, the carrier state, host animal reservoirs, animal-to-human transmission, human-to-human transmission, and environmental and physiological conditions that encourage propagation of microorganisms. Although significant data gaps exist in the complete characterization of the pathogenesis of *Cryptosporidium*, risk assessment approaches will enable health officials to communicate with water utilities, interpret water quality surveys, and define the adequacy of treatment in terms of acceptable public health risks (Rose *et al.*, 1997).

Dose-Response Studies

- In an experiment reported by DuPont *et al.* (1995), among 29 human subjects who were provided 30 or more oocysts, 62 % became infected. Acute illness lasted approximately 2.5 to 3.5 days with 4 to 11 loose stools produced per day. These findings suggest that human-to-human transmission of *C. parvum* is more likely to occur 2.5 to 3.5 days following infection in the primary case. Linear regression of the dose-response data indicated a human ID_{50} (the infectious dose causing disease in 50% of the population) of 132 oocysts. The authors concluded that a 'low' dose of *C. parvum* oocysts was sufficient to cause infection in healthy adults with no serologic evidence of past infection by this parasite (DuPont *et al.*, 1995).

- A number of dose-response studies using monkeys, gnotobiotic lambs and several strains of mice are presented in the 1994 *Cryptosporidium* Criteria Document. Casemore (1990) reported a 2- to 5-day incubation period for *C. parvum* and an excretion period of about 8 to 14 days in animals (species not identified). DuPont *et al.* (1995) reported that the ID_{50} for the Iowa strain of *C. parvum* oocysts necessary to infect neonatal mice was 60, or approximately half of the ID_{50} required to produce infection in humans (132 oocysts). The test strain of *C. parvum* in this case, however, was adapted to a mouse model prior to challenge studies, and this may account for the disparity in ID_{50} values. The relative similarity among infectious doses in mice and humans suggests that such mouse models are potentially useful in estimating certain human risks associated with cryptosporidiosis.

- Okhuysen *et al.* (1999) investigated the infectivity of three geographically diverse isolates (IOWA, UCP, and TAMU) of *C. parvum* genotype C in healthy adult volunteers. The TAMU isolate had significantly higher virulence, based on ID_{50} (9, 87, and 1042 oocysts for the TAMU, IOWA, and UCP isolates, respectively) and attack rate (86, 59, and 52% for TAMU, UCP, and IOWA, respectively). In addition, the mean time to onset of illness was shorter for the TAMU isolate (5 days vs. 9 to 11 days with the other two isolates), and a trend toward longer duration of diarrhea was observed in subjects infected with

the TAMU isolate (94.5 hours, compared to 81.6 and 64.2 hours for the UCP and IOWA isolates, respectively).

Environmental Factors

- As noted previously, *Cryptosporidium* oocysts are prevalent in surface waters and are less prevalent in ground waters. They are also found more often in waters in areas where animals such as cows are found, or where sewage runoff from urban areas occurs.

- Oocysts are resistant to a wide variety of environmental factors (e.g., temperature and chemical oxidation). As discussed previously in this report, this resistance, or hardiness, enables oocysts to survive outside the host for extended periods of time, thus increasing the chances for the organisms to encounter new hosts.

- The primary route of human infection by *C. parvum* involves ingestion of contaminated drinking water (Casemore, 1990). One of the primary difficulties in conducting risk assessments for *Cryptosporidium* arises from uncertainties associated with estimated levels of infectious oocysts in drinking water supplies. In addition, most detection methods for *Cryptosporidium* do not distinguish between viable and nonviable oocysts.

- Nahrstedt and Gimbel (1996) examined the influence of various factors contributing to the uncertainty in the determination of *Cryptosporidium* and *Giardia* concentrations in water samples. These factors were built into a statistical model, which was designed using experimental data, to provide more accurate estimates of oocyst/cyst concentration in a given water body once a sample from that body has been analyzed.

Epidemiologic Considerations

- The USEPA estimated in 1993 that approximately 155 million people may be exposed to *Cryptosporidium* in contaminated water every year. It is difficult to accurately estimate valid figures to describe the risk of acquiring cryptosporidiosis, for reasons such as the large number of unreported cases, the possibility of asymptomatic infections, and underestimated environmental levels (USEPA, 1994). Therefore, there is a disparity between the environmental occurrence data and the clinical data, as many unreported cases or asymptomatic cases go unnoticed.

- In the United States, the incidence of cryptosporidiosis often is estimated on the basis of surveillance data and reports of outbreaks that appear in the published literature. The CDC currently maintains an active surveillance system for cryptosporidiosis aimed at collecting information on both outbreaks and sporadic cases. While cryptosporidiosis is not a reportable disease in all states (CDC, 1994), it has been designated as notifiable at the national level since 1995 (CDC, 1998). It is important to note, however, that the CDC's surveillance of cryptosporidiosis is passive, in that the system is dependent upon a physician ordering a diagnostic test for *Cryptosporidium*. Most of this testing is done on adults who have AIDS and, as such, these surveillance data are not an adequate basis for estimating the true incidence of cryptosporidiosis in the United States.

- Groups at higher risk of exposure and infection to *Cryptosporidium* include secondary contacts of infected individuals, farm workers (Lengerich *et al.*, 1993), immunocompromised or immune-

suppressed individuals (Heald and Bartlett, 1994), and international travelers to regions where cryptosporidiosis is endemic.

- Groups that may experience more severe symptoms if infected with *Cryptosporidium* include children and immunocompromised or immune-suppressed individuals (Molbak *et al.*, 1994; Atherton *et al.*, 1995).

Risk Assessment Models

- Several risk models have been developed that assess the probability of cryptosporidiosis infection. These models are based upon assumptions concerning the levels of infectious oocysts in drinking water and upon the data generated from volunteer challenge studies. The estimated annual risk of waterborne cryptosporidiosis based upon these models ranges from 1 in 1,000 to 1 in 100,000 (Haas, 1994; Perz *et al.*, 1998).

- An exponential dose-response model developed by Haas (1994) was derived from studies in human volunteers conducted by DuPont *et al.* (1995) and Chappell *et al.* (1996), and also the Milwaukee cryptosporidiosis epidemic (Haas, 1994). This model describes the probability of infection (P_I) given exposure: $P_I=1-e^{-rN}$, where r represents the fraction of ingested oocysts that must survive to establish an infection and N is the daily exposure to oocysts (i.e., the concentration of oocysts in drinking water multiplied by the number of liters of water consumed in a day). According to the exponential model, *Cryptosporidium* exposure during the Milwaukee epidemic ranged from 0.6 to 1.3 oocysts per liter. Haas also applied the risk assessment model to consider data from previous water monitoring studies, and reported that the annual risk of contracting cryptosporidiosis in the United States may range from 4 in 1,000 to 1 in 100,000.

- Perz *et al.* (1998) applied a risk assessment approach to examine the role of tap water in waterborne cryptosporidiosis. The model was based upon the assumption that clinical infection results from exposure to a single oocyst. A theoretical *C. parvum* density in drinking water of 1 oocyst per 1,000 liters was used. The number of annual *Cryptosporidium* infections (I_j) was estimated as: $I_j = C \quad POP_j \quad Q_j \quad r_j$, where C is the concentration of *C. parvum* (oocysts/l of water), POP_j is the number of persons in the exposed subgroup, Q_j is the annual water intake in liters per year, j is the population subgroup (categorized by age and AIDS status), and r_j is single organism infectivity (infection/organism/person). The model was applied to derive the median annual risk of infection among immunocompetent individuals (1 in 1,000 probability using the assumed exposure level of 1 oocyst per 1,000 liters). The dominant parameter contributing to uncertainties in this risk assessment was oocyst concentration (e.g., a 10-liter sample volume for monitoring is too small to detect concentrations of 1 oocyst per 1,000 liters), suggesting that improvements in *Cryptosporidium* monitoring techniques will facilitate future risk assessment efforts.

- The usefulness of the ILSI Framework for microbial risk assessment was tested by Teunis and Havelaar (1999). They used the Framework to determine the human health risk of *C. parvum* in an urban population obtaining drinking water from a river. In the model, agricultural run-off and a sewage plant were contaminating sources and the water was treated conventionally (i.e., coagulation/flotation, and filtration and ozonation). Based on the model assumptions and data used, the median yearly individual risk of infection resulting from a well performing water treatment process was calculated as approximately 10^{-6}. The authors concluded that the ILSI Framework was a useful tool for defining

information needs and organizing available information in a consistent manner. Future research needs and suggestions for improving the framework were also discussed.

- Haas *et al.* (1996) used dose-response data on *Cryptosporidium* to establish waterborne concentrations of pathogen that led to various levels of risk. The concentration of oocysts in finished water for daily risks identical to a 1 in 10,000 annual risk of infection is 0.003/100L (95% confidence interval 0.0018 - 0.0064/100L).

Federal Regulations

- *Cryptosporidium* is regulated by the federal government as a primary drinking water contaminant. The federal regulatory activity associated with *Cryptosporidium* in drinking water was prompted by the 1996 Amendments to the Safe Drinking Water Act. The most significant promulgated and proposed rules are the Information Collection Rule (promulgated in 1996) (USEPA, 1996), the Interim Enhanced Surface Water Treatment Rule, and the Long Term I Enhanced Surface Water Treatment and Filter Backwash Rule.

- The Information Collection Rule required water utilities serving more than 10,000 people to test source water and finished water over an 18-month period (July 1997 to December 1998) (USEPA, 1996). The monthly testing included a variety of analytes including coliforms, turbidity, and *Cryptosporidium.* The rule was primarily a research effort and the USEPA is using the information for the development of future rules. The data generated from the Information Collection Rule is now available through Envirofacts (http://www.epa.gov/enviro/html/icr/icr_query.html).

- The Interim Enhanced Surface Water Treatment Rule, promulgated on December 16, 1998 (USEPA, 1998), applies to water utilities using surface water, or groundwater under the direct influence of surface water, and serving more than 10,000 people and was designed to establish physical removal efficiencies and to minimize *Cryptosporidium* levels in finished water. It set a maximum contaminant level goal (MCLG) of zero for *Cryptosporidium.* For systems that filter water during the treatment process, the rule requires a minimum 2-log *Cryptosporidium* removal efficiency. This rule includes *Cryptosporidium* in the watershed control requirement for unfiltered public water systems. The Agency estimates that as a result of the implementation of this rule, the likelihood of endemic illness from *Cryptosporidium* will decrease by 110,000 to 463,000 cases annually. The Agency believes that the rule also will reduce the likelihood of the occurrence of outbreaks of cryptosporidiosis by providing a larger margin of safety against such outbreaks for some systems.

- The Long Term I Enhanced Surface Water Treatment and Filter Backwash Rule was proposed April 10, 2000 (USEPA, 2000) and should be finalized by late Spring 2001. These provisions apply to smaller water systems (i.e., those serving less than 10,000 people) using surface water or groundwater under the direct influence of surface water systems. The requirements for the control of *Cryptosporidium* are similar to those of the Interim Enhances Surface Water Treatment Rule. The Long Term I Enhanced Surface Water Treatment provisions make *Cryptosporidium* a pathogen of concern for unfiltered systems, and such systems must comply with updated watershed control requirements. The Filter Backwash provisions will reduce the potential risks associated with recycling of contaminants removed during the filtration process. These provisions apply to all water systems that recycle water, regardless of population served. Physical removal is critical to the control of *Cryptosporidium* because it is highly resistant to standard disinfection practices.

VI. Analysis and Treatment

Analysis of Water Samples

Collection

- The current standard method for monitoring *Cryptosporidium* in water is EPA's Method 1622 (USEPA, 1999). This sample collection method relies on filtration using a capsule filter followed by immunomagnetic separation of the oocysts from the material captured. Before implementation of Method 1622, wound yarn filters were the most common filtration system in use; however, the use of capsule filters resulted in improved retention of oocysts. Calcium carbonate flocculation methods, which can concentrate up to 10 liters of water, have also been shown superior to wound yam filters but may interfere with viability determinations. Centrifugation-based concentration technologies such as vortex flow filtration, cross flow filtration, and continuous centrifugation could potentially recover greater numbers of oocysts than the currently used methods; however, they require interlaboratory validation. Flow cytometry also shows considerable recovery increases using either seeded or environmental samples. However, performance is influenced by water turbidity and composition.

Detection

- To determine oocyst concentrations, Method 1622 requires well slide staining using fluorescently labeled monoclonal antibodies and 4',6-diamidino-2-phenylindole (DAPI), and the cells are visualized by fluorescence and differential interference contrast (DIC) microscopy (USEPA, 1999). Several applications of polymerase chain reaction (PCR) technology have been described for the detection of *Cryptosporidium*, some of which may be able to distinguish viable from nonviable oocysts; however, enzymatic inhibition remains problematic. Laser scanning devices have also performed well in early studies. More research is required on this technology.

- Since the determination of *Cryptosporidium* viability is critical in assessing the public health threat of cryptosporidiosis, a number of viability assays have been described and compared to animal infectivity models. Some viability assays (e.g., *in vitro* excystation and vital dye staining) have produced conservative estimates of oocyst viability when compared to animal modeling data. Limitations in viability assays have precluded their routine use in environmental samples (Black *et al.*, 1996; Belosevic *et al.*, 1997; Jenkins *et al.*, 1997).

Analysis of Biological Samples

- The 1994 *Cryptosporidium* Criteria Document described the increased sensitivity of immunofluorescent antibody-based (IFA) procedures, although traditional staining methods such as the Ziehl-Neelsen stain are still widely used. Enzyme immunoassay (EIA) methods are fast, inexpensive, easily performed, and show sensitivity approaching that of immunofluorescence methods. However, a lack of confirmatory analyses may preclude their routine use. Several molecular techniques including PCR based methods have also been developed but are not yet widely used (Filkorn *et al.*, 1994; Johnson *et al.*, 1995).

Drinking Water

Removal of Cryptosporidium

- Of the technologies available to the drinking water industry, membrane processes (forms of micro- and ultra-filtration) appear to provide the most significant levels of *Cryptosporidium* removal. Conventional treatment practices appear capable of meeting 2-log removals in most of the cases studied to date. Although direct filtration and in-line filtration may be expected to be less effective than conventional treatment, this has not yet been demonstrated in a conclusive manner. Alternative technologies such as diatomaceous earth filtration and slow sand filtration appear capable of achieving comparable, if not better, levels of *Cryptosporidium* removal than conventional treatment. A comparison of removal efficiencies of some bench-, pilot-, and full-scale water treatment processes is presented in Table 3 below.

Table 3. *Cryptosporidium* Removal Efficiencies for Selected Physical and Chemical Processes

Treatment Process Description	Removal Achieved (log)		
	Bench Scale	Pilot Scale	Full Scale
Coagulation + Gravity Settling	< 1.0[a]	1.4 - 1.8[b]	0.4 - 1.7[g]
Coagulation + Filtration		2.7 - 5.9[b]	1.6 - 4.0[e]
		2.5 - 3.8[h]	
		2.7 - 2.9[i]*	
Coagulation + Gravity Settling + Filtration		4.2 - 5.2[b]	1.6 - 4.0[e]
		> 5.3[f]	< 0.5 - 3.0[f]
		2.1 - 2.8[i]*	1.0 - 2.5
Coagulation + Dissolved Air Flotation	2.0 - 2.6[a]		
Slow Sand Filtration		> 3.7[c]	
Diatomaceous Earth Filtration		> 4.0[c]	
Coagulation + Microfiltration		> 6.0[d]	
Ultrafiltration		> 6.0[d]	

* Range of average removal efficiencies based on reservoir and river water sources.
Source: Adapted from Frey *et al.* (1998)
References cited by Frey *et al.* (1998): [a] Plummer *et al.*, 1995; [b] Patania *et al.*, 1995; [c] Schuler *et al.*, 1988; [d] Jacangelo *et al.*, 1995; [e] Nieminski and Ongerth, 1995; [f] LeChavallier *et al.*, 1991; [g] Kelley *et al.*, 1994; [h] Anderson *et al.*, 1996; and [i] Nieminski, 1995.

Inactivation of Cryptosporidium

- Ozone appears to be the best chemical disinfectant for *Cryptosporidium* inactivation (Korich, *et al.*, 1990; Finch *et al.*, 1997), and chlorine dioxide is the second most effective disinfectant (Peeters, *et al.*, 1989; Korich, *et al.*, 1990; Finch *et al.*, 1997, Liyanage, *et al.*, 1997a). Mixed oxidant and ultraviolet light systems appear to be promising, but have only been tested in minimal fashion when compared with ozone (Venczel, *et al.*, 1997; Campbell, *et al.*, 1995; Arrowood, *et al.,* 1996). Also holding some promise are the sequential disinfection systems of ozone followed by chlorine and ozone followed by monochloramine (Liyanage *et al.*, 1997b, Finch *et al.*, 1997).

VII. Research Requirements

- Frey *et al.* (1998) evaluated the current state of *Cryptosporidium* research, determined the gaps in the data, and assessed future research needs. This section presents some of the existing needs for research.

Source Water Occurrence

- The source and occurrence of *Cryptosporidium* in watersheds has been characterized, although continued improvements in monitoring methods and analytical techniques would increase our understanding of these issues. Research to discover specific contamination sources also would contribute to public health protection.

Health Effects

- Continued research in drug therapy is important in optimal treatment of *Cryptosporidium*. There has been very little progress in elucidating the pathogenic mechanisms involved in cryptosporidiosis, although the EPA-sponsored human infectivity studies should provide useful information.

Risk Assessment

- More information is needed to better identify and characterize outbreaks, to assess the risks to susceptible populations, and to identify the infectious dose and virulence of *Cryptosporidium* across different populations. In addition, better diagnostic serological methods need to be developed, validated, and more serology-based epidemiology studies need to be completed. Risk assessment also would be improved by calibration of risk assessment models to make them more precise.

Analysis

- Detection methods continue to be quite variable and the need still exists for a standard method that is accurate, precise, quick and affordable. Many of the newer technologies have not been sufficiently validated outside the laboratory. The analysis of large sample volumes still presents a challenge for detection. In addition, not enough is known about the basic cell biology of *Cryptosporidium*. Greater knowledge in this area will not only help in the development of an accurate detection method, but it will also advance the improvement of viability, infectivity, and speciation assays for environmental *Cryptosporidium*. Finally researchers are still faced with the challenge of overcoming interferences posed by environmental samples for molecular-based techniques.

Drinking Water Treatment

- There is a great need for development and evaluation of possible/optimal methods for disinfection and removal of *Cryptosporidium* (e.g., ozonation, UV, improved filtration). In addition, due to concerns associated with chlorination byproducts, compounds other than chlorine should be sought as residual disinfectants in finished drinking water supplies. Complete evaluation of treatment for oocyst removal is dependent on better detection methods and more rigorous enumeration practices. Other gaps in the data regarding treatment of drinking water include the usefulness and efficacy of surrogates to determine success of treatment, the impact of the treatment process on oocyst viability and survival at the molecular level, and guidelines or a decision matrix to assist in treatment selection.

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