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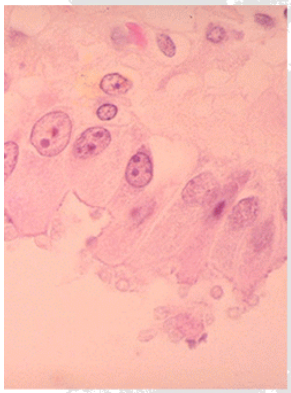
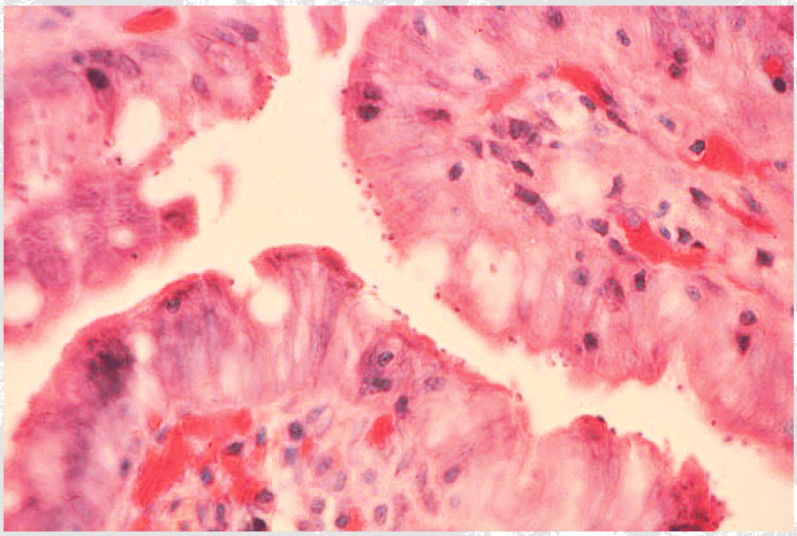
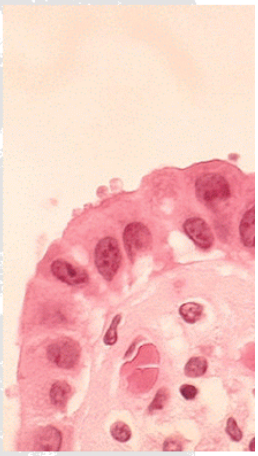
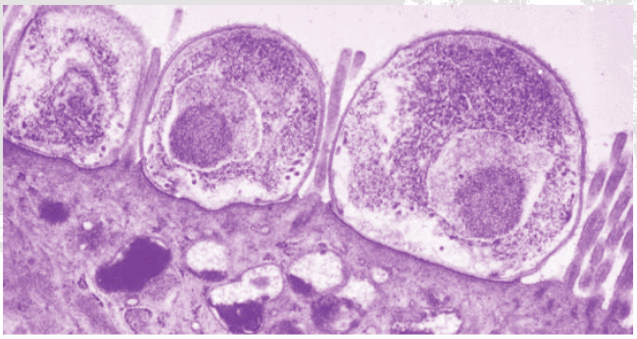
Cryptosporidium: Human Health Criteria Document









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EXECUTIVE SUMMARY

The Safe Drinking Water Act requires the U.S. Environmental Protection Agency (EPA) to publish regulations to control disease-causing organisms (pathogens) and hazardous chemicals in drinking water. One of the regulations published by EPA to control pathogens is known as the Surface Water Treatment Rule (54 FR 27486; June 29, 1989). The intent of this rule was to control *Giardia*, pathogenic viruses, and *Legionella*, all of which have caused many outbreaks and cases of waterborne illness.

Another prominent waterborne pathogen is the protozoan *Cryptosporidium*. This organism has caused a number of waterborne disease outbreaks in the U.S. and other countries. In 1994, EPA prepared a literature review of the published data on *Cryptosporidium*, entitled "*Cryptosporidium* Criteria Document," to establish a basis for a regulation to control this organism. The following document, "Drinking Water Criteria Document Addendum: *Cryptosporidium*," updates the 1994 publication. It includes new information available in the literature from 1994 to the present and was prepared to support EPA's Interim Enhanced Surface Water Treatment Rule, which has as a primary focus the control of *Cryptosporidium*. The update provides information on general characteristics of *Cryptosporidium*, its occurrence in human and animal populations and in water, the health effects associated with *Cryptosporidium* infection, outbreak data, and an assessment of risk. The document also includes information about analytical methods to enumerate *Cryptosporidium* in water and the effectiveness of various water treatment practices in its removal.

The document demonstrates that *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 U.S., most notably in Milwaukee, where an estimated 400,000 people became ill. The document shows that 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 the information presented in the 1994 document and in the following update is sufficient 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.

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I. Introduction

The United States Environmental Protection Agency (USEPA) Office of Water is preparing and revising the health criteria documents that will support the Phase I Disinfectant/Disinfectant Byproduct (DBP) Rule, the Interim Enhanced Surface Water Treatment Rule (IESWTR) and the Groundwater Disinfection Rule (GWDR). As part of the rule making process, the USEPA is required to compile a complete and current compendium of the information used as criteria to support creation of the rules. The first step in this process occurred in June of 1994 with the preparation of the USEPA Draft Drinking Water Criteria Document for *Cryptosporidium,* hereafter referred to as the "1994 *Cryptosporidium* Criteria Document." This addendum provides an update to supplement (but not duplicate) the 1994 *Cryptosporidium* Criteria Document. This addendum uses the same table of contents formatting as the 1994 document to facilitate cross referencing between the two documents. Much of the published research since the 1994 document has focused on the speciation of *Cryptosporidium,* better methods to detect *Cryptosporidium* in the environment, and improvements in water treatment technology. Consequently, this addendum includes much new information regarding speciation and improvements in analyses and treatment. The overall objective is to provide a comprehensive *Cryptosporidium* information resource to Federal, State, and local health officials responsible for protecting public health and the environment.

II. General Information and Properties

A. History and Taxonomy

1. History

Cryptosporidium was described by Tyzzer in 1907 but was considered 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). However, the diagnosis of cryptosporidiosis in humans in 1976 and the subsequent connection of

Cryptosporidium to epidemic waterborne disease have since fostered worldwide interest in the study of this microorganism. By the time the Centers for Disease Control and Prevention implemented routine reporting of *Cryptosporidium* among AIDS patients in 1982, only 13 cases of human cryptosporidiosis had been documented (Ungar, 1990). Since 1982, more than 1,000 reports of human cryptosporidiosis have been documented in almost 100 countries, reaching all continents with the exception of Antarctica (Fayer, 1997). At the time of this writing, it is estimated that the annual number of cryptosporidiosis cases exceeds several million worldwide (Casemore *et al.*, 1997).

Cryptosporidium was first recognized as a waterborne pathogen during an outbreak in Braun Station, Texas, where more than 2,000 individuals were afflicted with cryptosporidiosis (D'Antonio *et al.*, 1985; Graczyk *et al.*, 1998b). Since that time, outbreaks affecting over a 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). A complete history of the waterborne outbreaks of cryptosporidiosis is provided in section III-F.

2. 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. More than 20 species have been described based upon the hosts from which they were originally isolated (a complete list is included in Table II-1, 1994 *Cryptosporidium* Criteria Document). By 1997, however, interspecies transmission studies, morphological evaluations and immunological analyses had reduced this number to eight valid species (Fayer *et al.*, 1997a). Since 1997, two other species have been identified, brining the total number of valid species to ten. *Cryptosporidium saurophilum* was isolated from populations of lizards, Schneider's skink (*Eumeces*

schneideri), and desert monitors in Australia (Koudela and Modry, 1998). *Cryptosporidium andersoni* was recovered from the feces of domestic cattle, *Bos taurus* (Lindsay *et al.*, 2000). Table 1 lists the ten valid *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, 1997; Fayer *et al.*, 2000). Genetic research has provided support for the species *C. felis* and *C. wrairi*, whose distinctness from other *Cryptosporidium* species had been previously questioned (Bornay-Llinares *et al.*, 1999; Morgan *et al.*, 1999b; Morgan *et al.*, 1999c; Morgan *et al.*, 1998b; Sargent *et al.*, 1998; Xiao *et al.*, 1999a; Xiao *et al.*, 1999b).

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)

O'Donoghue (1995) reported that infection caused by *Cryptosporidium* had been observed in 79 mammalian species (including humans) in addition to numerous reptilian, amphibian, avian, and fish hosts. Fayer *et al.* (2000) documented *Cryptosporidium* infection in more than 150 mammalian species. Illness in humans,

however, is confined primarily to infections associated with *C. parvum* (O'Donoghue, 1995). A single case of human cryptosporidiosis in an immunocompromised individual was attributed to *C. baileyi* (Ditrich, 1991), but this organism was later shown to be *C. parvum* (Fayer, pers. comm.). Two recent studies have reported *C. felis* infections in HIV-positive patients in the United States (Morgan *et al.*, 2000a; Pieniazek *et al.* 1999). In addition, *C. meleagridis* was detected from an HIV-infected individual in Kenya (Morgan *et al.*, 2000a).

The taxonomy of *Cryptosporidium* is in the forefront of current research on the parasite, and changes in nomenclature may be expected. 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; Morgan *et al.*, 1999a; Morgan *et al.*, 1999b; Morgan *et al.*, 1999c; Morgan *et al.*, 1999d; Morgan *et al.*, 1998a; Spano *et al.*, 1998a; Spano *et al.*, 1998b; Sulaiman *et al.*, 1998; Xiao *et al.*, 1999a; Xiao *et al.*, 1999b).

Several studies have suggested the possibility of distinct transmission cycles among different genotypes (Awad-El-Kariem, 1999; Awad-El-Kariem *et al.*, 1998; Morgan *et al.*, 1998a; Patel *et al.*, 1998; Peng *et al.*, 1997; Sulaiman *et al.*, 1998; Widmer *et al.*, 1998c). Alternatively, multiple genotypes may be able to circulate among different host species, and mixed infection with genotypically different populations may arise through selection in different host environments (Widmer *et al.*, 1998b). Nevertheless, some researchers have suggested that these genotypes should be considered to be separate species (Morgan *et al.*, 1999c; Xiao *et al.*, 1999b).

Until recently, it was not possible to assess genetic variation among *Cryptosporidium* isolates. Molecular studies utilizing restriction fragment length polymorphism (RFLP) analysis, isoenzyme electrophoresis, and

arbitrarily primed polymerase chain reactions (AP-PCR) have helped to characterize these subtle differences among individual isolates; these recent studies are described below.

One study using PCR and restriction mapping suggested that differences in the genetic sequences within the 18S ribosomal RNA (rRNA) region of *Cryptosporidium* could be used to distinguish individual species (*C. muris*, *C. parvum*, and *C. baileyi*) and could assist in the development of taxonomic classification (Awad-El-Kariem *et al.*, 1994). Webster (1993) applied a battery of molecular taxonomic methods (flow cytometry, PCR, and RFLP) to detect and classify *Cryptosporidium* oocysts from geographically diverse isolates. The isolates exhibited genetic homogeneity for the most part, although differences in isoelectric points and restriction maps indicated genetic differences among *C. parvum* isolates from humans and bovines.

Isoenzyme electrophoresis studies (O'Donoghue, 1995; Awad-El-Kariem, 1995; Awad-El-Kariem *et al.*, 1998) have been applied to characterize animal and human oocyst isolates of *C. parvum* from different geographical locations. The discovery of two unique isoenzyme forms indicates the existence of separate subpopulations within the *C. parvum* species, one which infects primarily humans and the other which infects animals. Follow-up studies using AP-PCR and isoenzyme typing (Carraway *et al.*, 1994; Awad-El-Kariem *et al.*, 1996; Awad-El-Kariem *et al.*, 1998 have confirmed the two unique profiles among *C. parvum* isolates corresponding to the animal and human types. Cross-transmission infection studies performed by Awad-El-Kariem *et al.* (1996, 1998) indicated that most human isolates were not capable of establishing infection in a murine model, whereas all animal isolates were infectious in mice, supporting the existence of genetically distinct populations of this strain.

Analysis of genetic polymorphisms among *C. parvum* isolates from nine human outbreaks and from several bovine sources (Peng *et al.*, 1997) indicated the existence of two genotypes with genetic differences among adhesion proteins. Genotype 1 was observed exclusively in human isolates and has been called the human or H genotype. Genotype 2 was observed both in calf isolates and in isolates from human patients who reported direct or indirect exposure to infected cattle, and this genotoype has been called the cattle or C genotype. These findings support two distinct transmission cycles of *C. parvum* in humans: (1) human to human, and (2) animal to human. This hypothesis is supported by the work of Carraway *et al.* (1997), who conducted RFLP analyses on *C. parvum* oocysts isolated from humans and cows. While all calf isolates exhibited genetic homogeneity at a specific 2.8-kb fragment, human isolates exhibited multiple profiles at this locus: one found exclusively in humans, and one with a superposition of both profiles, indicative of heterogeneity among parasite populations.

Sequence and/or PCR-RFLP analyses of various loci have confirmed the genetic distinctness of the human and cattle genotypes. The examined loci include the 18S small subunit (SSU) rRNA gene (Morgan *et al.*, 2000a; Xiao *et al.*, 1999a; Xiao *et al.*, 1999b), ribosomal ITS1 and ITS2 (internal transcribed spacer) regions (Morgan *et al.*, 1999a), the acetyl-CoA synthetase gene (Morgan *et al.*, 2000a; Morgan *et al.*, 1998a), the COWP (*Cryptosporidium* oocyst wall protein) gene (Patel *et al.*, 1998; Spano *et al.*, 1997), the *dhfr* (dihydrofolate reductase) gene (Morgan *et al.*, 1999b), the TRAP-C1 and TRAP-C2 (thrombospondin-related adhesive protein of *Cryptosporidium*) genes (Spano *et al.*, 1998b; Sulaiman *et al.*, 1998), and the HSP-70 (heat shock protein) gene (Morgan *et al.*, 2000a). The genetic distinctness of the two genotypes also was supported by a multilocus study performed by Spano *et al.* (1998a) on 28 isolates of *C. parvum* originating from Europe, North and South America, and Australia. The study analyzed the poly(T) (polythreonine) gene, COWP gene, TRAP-C1 gene, and RNR (ribonucleotide reductase) gene by using PCR-RFLP, as well as the ITS1 region using a genotype-

specific PCR. All isolates clustered into two groups, one comprising isolates of both human and animal origin and the other comprising only human isolates, and no recombinant genotypes were found. Khramtsov *et al.* (2000) demonstrated that two virus-like double-stranded (ds) RNAs are present in *C. parvum.* Although the dsRNA sequences were similar in isolates of either human or calf origin, slight but consistent differences in nucleotide sequences at select sites were noted between the two genotypes.

While researchers have demonstrated substantial genetic differences between the human and cattle genotypes, some studies also have found variation within these genotypes. Widmer *et al.* (1998a) reported evidence of polymorphisms within the human genotype and of recombination between the human and cattle genotypes, based on sequence and PCR-RFLP analysis of the -tubulin intron. In a separate study (Widmer *et al.*, 1998b), sequence and PCR-RFLP analyses of the -tubulin intron also revealed polymorphisms within the human and cattle genotypes, with sequences indicative of interallelic recombination in two isolates. Caccio *et al.* (2000) provided further evidence that the human and cattle genotypes are not genetically homogeneous. Sequence analysis of a locus containing microsatellite repeats in 94 *C. parvum* isolates demonstrated heterogeneity in both the human and cattle genotypes. Two subgenotypes of the human genotype and four subgenotypes of the cattle genotype were identified, but the prevalence and significance of these intragenotype differences are not clear. For example, Okhuysen *et al.* (1999) demonstrated that three different *C. parvum* isolates of the cattle genotype differed in their infectivity for humans.

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

1998a; Pereira *et al.*, 1998; Xiao *et al.*, 1999b). A genetically distinct strain referred to as the mouse genotype has been identified in *C. parvum* isolates from mice in Australia, the United States, the United Kingdom, and Spain by using sequence analysis of the 18S SSU rRNA, ITS1 and ITS2, *dhfr*, HSP-70, COWP, and acetyl CoA loci, as well as RAPD (random-amplified polymorphic DNA analysis) (Morgan *et al.*, 1999a; Morgan *et al.*, 1999b; Morgan *et al.*, 1999c; Morgan *et al.*, 1999e; Morgan *et al.*, 1998a; Xiao *et al.*, 1999b).

Another genetically distinct strain referred to as the pig genotype has been identified by sequence analysis of the 18S SSU rRNA, ITS1 and ITS2, *dhfr*, and acetyl CoA loci, as well as RAPD and AP-PCR, of *C. parvum* isolates from pigs in Switzerland, the United States, and Australia (Morgan *et al.*, 1999a; Morgan *et al.*, 1999b; Morgan *et al.*, 1999c; Morgan *et al.*, 1999f; Morgan *et al.*, 1998a; Pereira *et al.*, 1998; Xiao *et al.*, 1999b). A marsupial genotype also has been suggested based on genetic analysis of *C. parvum* isolates from a koala from South Australia and a red kangaroo from Westem Australia (Morgan *et al.*, 1999a; Morgan *et al.*, 1999b; Morgan *et al.*, 1999c; Xiao *et al.*, 1999b). Sequence analysis of the 18S SSU rRNA, ITS1 and ITS2, and *dhfr* loci, as well as RAPD, of these isolates has confirmed their distinctness from all other genotypes of *C. parvum.*

The dog genotype has been identified from sequence analysis of the 18S SSU rRNA and HSP-70 loci, which demonstrated genetic distinctness in *C. parvum* isolates from dogs from Australia and the United States (Morgan *et al.*, 2000b; Morgan *et al.*, 1999c; Xiao *et al.*, 1999b). The dog genotype also has been isolated from HIV patients (Pieniazek *et al.*, 1999). Xiao *et al.* (1999b) found genetically distinct strains in *C. parvum* isolates from a ferret and a monkey using sequence analysis of the 18S SSU rRNA gene, suggesting the possibility of a ferret genotype and a monkey genotype. Although the occurrence of genetically distinct strains

of *C. parvum* may warrant a taxonomic revision of the genus, scientists have expressed concern over designating a new species based on a small number of base pair differences (USEPA, 1999a).

Some researchers have supported the hypothesis that *Cryptosporidium* may belong to a clonal population structure, based on correlations between phenotypic and genotypic markers and the widespread occurrence of identical genotypes (Awad-El-Kariem, 1999; Morgan *et al.* 1999c). The essential concept of the clonal hypothesis is that different strains of the same species may clone (or propagate) different forms of the same gene due to geographic isolation. As a consequence of this isolation, mutated or otherwise modified genetic loci will become perpetuated in a clonal manner within the species in the absence of sexual reproduction. Other researchers have questioned the clonal population hypothesis on the basis of mixed infection and apparent genetic recombination (Patel *et al.*, 1998; Widmer *et al.*, 1998a; Widmer *et al.*, 1998b). Studies are underway to further elucidate the population structure of *Cryptosporidium.*

B. Life Cycle

A complete description of the life cycle of *Cryptosporidium* is provided in the 1994 USEPA *Cryptosporidium* Criteria Document (Figure II-1, p. II-5). *Cryptosporidium* is excreted in the feces of an infected host in the form of an oocyst, which represents the only exogenous stage of the life cycle. The oocyst consists of four sporozoites housed within a sturdy, multi-layered wall. The thick-walled oocyst is the environmentally resistant form of the parasite, resulting from the fertilization of macrogametes within the host, and it is appreciably resistant to natural decay in the environment as well as to most disinfection processes. 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 new host's gastrointestinal tract.

C. Morphological Features

A complete description of the morphological features of each life cycle stage (oocyst, sporozoite, trophozoite, merozoite, microgametocyte, macrogametocyte) of *Cryptosporidium* is provided in the 1994 *Cryptosporidium* Criteria Document (p.II-7–II-9). 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 they are probably artifactual. As a result, the researchers suggested that the apparent fold no longer be considered a diagnostic feature of *Cryptosporidium parvum.*

D. Species Transmission

1. Direct Transmission Between Humans

A number of studies show that person-to-person transmission of cryptosporidiosis infection can occur within families, day care centers, hospitals, and in urban environments where population densities are high (USEPA, 1994). The route of infection follows one of two paths: direct, through fecal-oral contact, or indirect, through fomites (inanimate objects). Casemore (1990) evaluated cryptosporidiosis associated with nosocomial (hospital acquired) transmission as well as its association with traveller's diarrhea. In the hospital setting, cryptosporidiosis may be spread from one patient to another or from a patient to a staff member. *Cryptosporidium* also is a primary cause of traveller's diarrhea, typically being transmitted through contaminated water or food (Casemore, 1990). Transmission is affected by ethnic and dietary differences (e.g., Muslims exhibit a lower prevalence than many other ethnic groups).

After visitors who came to Milwaukee during the 1993 outbreak returned to their homes, 74 members of their households who had not accompanied them on the visit tested positive for *Cryptosporidium* (Mackenzie *et al.*, 1995b). Five percent of these infected household members ultimately developed cryptosporidiosis, thereby meeting the definition of secondary transmission within the household. Osewe *et al.* (1996) also evaluated data following the Milwaukee outbreak and concluded that secondary transmission rates to household members during the epidemic were comparatively low (3-5%). In addition to the person-to-person transmission, there appeared to be a recurrence of infections that were acquired by the Milwaukee visitors during the outbreak. Transmission to susceptible individuals in the Milwaukee area continued after the massive initial outbreak, but decreased rapidly within the two months that followed.

Cordell and Addiss (1994) tracked cryptosporidiosis in various child care settings and observed a 12 to 22% rate of secondary spread between children to other household members. Newman *et al.* (1994) reported household transmission of *C. parvum* infection in an urban community in northeast Brazil. In this study, 18 of 31 (58%) households, whose members ranged in age from 5 months to 47 years, showed at least one secondary case of cryptosporidiosis (identified either by stool examination or serologic testing). Secondary cases involved a total of 30 persons, yielding an overall transmission rate of 19%. Of the 202 persons included in this study, 94.6% had evidence of serum IgG or IgM antibodies to *Cryptosporidium*, demonstrating that a significant rate of person-to-person transmission of *Cryptosporidium* may occur.

The rate of transmission between immunocompromised individuals may be high. In group homes housing HIV-positive individuals, Heald and Bartlett (1994) reported a high (not specified) rate of transmission among occupants. Lopez-Velez *et al.* (1995) found an overall prevalence of intestinal cryptosporidiosis of 15.6% in

AIDS patients. However, the rate among homosexual partners was higher (33.3%) than among intravenous drug users (10.6%), strongly suggesting person-to-person transmission of cryptosporidiosis. Sears *et al.* (1994) concluded that strict infection control measures must be followed especially in crowded living conditions and where immunocompromised persons reside.

Secondary transmission of cryptosporidiosis has been observed among humans whose occupation places them near primary cases within a confined space. 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). Of 50 crew members, 62% exhibited symptoms of cryptosporidiosis, and oocysts were detected in stool samples of 10 individuals (20%). In such an outbreak, distinguishing between primary infections (i.e., due to ingestion of contaminated water) and secondary infections (i.e., due to fecal-contaminated fomites, food, or other infected individuals) is difficult because the individuals involved were obviously in a closed environment where frequent contact between humans occurs. In some instances it may not be possible to determine whether transmission between humans is the primary cause of cryptosporidiosis, especially when humans also come into contact with animals through occupational or recreational activities (Adam *et al.*, 1994).

2. Transmission Between Animals and Humans

The 1994 USEPA *Cryptosporidium* Criteria Document provided adequate evidence for the transmission of *Cryptosporidium* from animals, particularly livestock, to humans. Domestic animals such as calves and lambs are common zoonotic reservoirs involved in occupational exposure, indirect zoonotic transmission, and contamination of food (e.g., sausages, offal, and raw milk). Animals may also contribute to environmental contamination in sources such as watersheds, food crops, and recreational waters. For example, 70 cases of

cryptosporidiosis in the U.K. resulted from exposure to a single swimming pool with water contaminated by animal excreta. Pets, however, have not often been implicated as a source of infection and are not considered a major risk factor for acquisition of cryptosporidiosis (Glaser, 1998).

Of ten valid species infecting all vertebrate groups, only one, *C. parvum*, represents a global public health problem due to its zoonotic potential (Graczyk, 1998b). Although infections in humans primarily have been attributed to *C. parvum*, a single case of *C. baileyi* infection in an immunosuppressed individual has been reported (Ditrich, 1991). Two instances of *C. felis* infections in HIV-positive patients in the United States are known (Morgan *et al*., 2000a; Pieniazek *et al*. 1999). In addition, *C. meleagridis* was detected from an HIV-infected individual in Kenya (Morgan *et al*., 2000a).

A number of studies have been conducted to determine if *C. parvum* can cross species lines to non-mammalian species. Graczyk *et al*. (1996a) attempted to infect a variety of fish, amphibia and reptiles with *C. parvum* without success. Although the oocysts were present in the cloaca of two fish and one lizard post-exposure, no life-cycle stages were detected in histological sections taken from any of the inoculated species. From these results, the authors concluded that the oocysts were unable to establish an infection in the gastrointestinal tract of lower vertebrates even though the inoculated *C. parvum* oocysts were retained by these animals for at least 14 days following ingestion. Although animal excreta have been shown to contaminate watersheds and source waters linked to outbreaks (see Section III-C), evidence of direct animal transmission of *Cryptosporidium* to humans is limited to a few examples (Adam, 1994; Casemore, 1990). The search for the source of *Cryptosporidium* in sporadic cases has been just as elusive. To assess infectious risks associated with pet ownership, Glaser *et al*. (1998) conducted a case-control study of HIV-infected individuals with and without cryptosporidiosis. No statistically significant differences were observed in the rate of overall pet ownership, cat

ownership, or bird ownership between the two groups, and only a slight correlation between dog ownership and human cryptosporidiosis was noted. The authors concluded that pets do not represent a major risk factor for acquisition of cryptosporidiosis.

Despite the strict host-specificity of a number of mammalian species of *Cryptosporidium*, the discovery that a *C. parvum*-refractory host (a host which ingests infectious oocysts but is not susceptible to infection) can excrete intact oocysts has raised the issue that, if oocyst infectivity is retained during travel through the intestinal tract, the refractory host could serve as a mechanical vector for dissemination of the parasite through the environment (Graczyk, 1998b). In fact, research with some avian species has proven that oocysts do retain their infectivity after passing through the intestinal tracts of the animals. Insects have been shown to carry *C. parvum* oocysts on their outer surfaces as well as in their intestinal tracts. The following paragraphs briefly summarize relevant findings about insects and birds as vectors for *Cryptosporidium.*

The contribution of migratory waterfowl to the overall public health risk of cryptosporidiosis remains unclear. Transport of oocysts through migratory waterfowl has been demonstrated. Viable *Cryptosporidium* oocysts have been found in fecal samples and cloacal lavages of gulls which fed on sewage or other refuse (Smith *et al.*, 1993). 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.*, 1996c; Graczyk *et al.*, 1997a). In another study, *C. parvum* oocysts which were recovered from goose fecal samples collected in the Chesapeake Bay successfully infected laboratory mice (Graczyk *et al.*, 1998c). The epidemiological implications of these findings should be considered, especially in areas near reservoirs or in waters where shellfish are harvested and may be consumed raw.

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.

E. Summary

Cryptosporidium has a complex life cycle that involves numerous developmental stages culminating in the production of oocysts that are resistant to adverse environmental conditions. Ten species are currently recognized within the genus; however, recent molecular research may warrant taxonomical revision including the addition of new species. *Cryptosporidium* infection has been documented in over 150 mammalian species. At least one genotype within *C. parvum* appears to be transmitted exclusively through humans. There is sufficient evidence that secondary *Cryptosporidium* infections can occur in humans following primary infection from ingestion of contaminated drinking water. Human-to-human transmission occurs through the fecal-oral route and reinfections are common where human density is high or among people living in close quarters. *C. parvum* exhibits specificity with regard to infection in mammals. Lower vertebrates such as fish, frogs, and lizards are not susceptible to infection. Other than *C. parvum*, only *C. felis* and *C. meleagridis* have been associated with human cryptosporidiosis. Species that infect other mammals, such as *C. wrairi*, may be

genetically linked to *C. parvum*; however, the public health significance of this relationship concerning potential transmission of *C. wrairi* among human populations is not known.

III. Occurrence

A. Worldwide Distribution

1. Distribution in Animal Populations

Although the 1994 *Cryptosporidium* Criteria Document acknowledged that members of the genus *Cryptosporidium* have been identified in many animal species, it focused primarily on the prevalence of *Cryptosporidium* in domestic animals (cattle, lambs, pigs, goats, deer, and horses) and house pets (dogs, cats, hamsters, guinea pigs, and rabbits). Prior to the writing of the 1994 document, and since that time, *Cryptosporidium* has been identified in numerous mammalian, avian, reptilian, and piscine hosts worldwide. One frequently cited review provided an extensive list of the animals in which *Cryptosporidium* has been detected (O'Donoghue, 1995). The findings of this review article are summarized below.

- *Cryptosporidium* infections have been recorded in 79 mammalian species (including humans). Natural infections (i.e., infections not induced in an experimental setting) have been described in domestic, wild, and captive mammals with the majority occurring in farm, zoo, pet, and lab animals. Most infections in mammals have been attributed to *C. parvum*; however, some natural infections in mice, rats, cattle, mountain gazelles, and a camel have been attributed to *C. muris*.

- *Cryptosporidium* infections have been detected in over 30 species of birds primarily in domestic flocks or aviary birds, but also in wild bird populations. Two species of *Cryptosporidium* species are currently considered as valid pathogens in birds (*C. meleagridis* and *C. baileyi*; see Table 1, section II-A), and infections have been attributed to both, although neither species has demonstrated host specificity.

- *Cryptosporidium* infections have been reported in over 57 different reptilian species including 40 species of snakes, 15 species of lizards, and 2 species of tortoises. *Cryptosporidium serpentis* is currently the only valid named species of *Cryptosporidium* known to cause infections in reptiles. Previous descriptions of other species causing infections in reptiles (*C. crotali, C. lampropeltis, C. ameivae and C. ctenosauris*) are consistent with the morphology of another parasite genus (*Sacrocystis* spp.) and therefore are considered invalid (Fayer, 1997).

- *Cryptosporidium* infections have been detected in nine species of fish. Infections have been observed in both freshwater and marine species, and in cultured, captive, ornamental, and wild-caught fish. The only valid named species of *Cryptosporidium* causing infection in fish is *C. nasorum*, named after the first reported infection in a tropical marine fish (*Naso lituratus*) (Hoover *et al.*, 1981).

- In addition to the mammalian, avian, reptilian, and piscine hosts mentioned above, *Cryptosporidium* infections have been identified in two amphibian species, *Ceratophrys ornata* (Bell's horned frog; Crawshaw and Mehren, 1987) and *Limnodynastes tasmaniensis* (spotted grass frog; O'Donoghue and Mirtschin, unpublished), and in one invertebrate species, *Ruditapes decussatus* (Portuguese clam; Azevedo, 1989).

Additional hosts reported in the literature, but not mentioned in the O'Donoghue (1995) review article, include Fell Pony foals (Scholes *et al.*, 1998), muskrat (Petri *et al.*, 1997), African hedgehog (Graczyk *et al.*, 1998a), dugong (Hill *et al.*, 1997), slow loris, white rhinoceros, Indian elephant, Thorold's deer (Majewska *et al.*, 1997), and iguana (Fitzgerald *et al.*, 1998). Fayer *et al.* (2000) documented *Cryptosporidium* infection in more than 150 mammalian species.

2. Distribution in Human Populations

The distribution of *Cryptosporidium* in human populations is worldwide, occurring in both developed and under-developed countries, urban and rural areas, and in temperate as well as tropical climates (Fayer, 1997; O'Donoghue, 1995). The 1994 *Cryptosporidium* Criteria Document described the worldwide distribution of human cryptosporidiosis in 45 different countries. Since 1982, human cryptosporidiosis has been documented in 95 countries and on every continent except Antarctica (Fayer, 1997). A list of these countries, including the 45 listed in the 1994 document, is given in Table 2.

Worldwide prevalence rates of human cryptosporidiosis from the most recent compilations of coprologic (stool) and serologic (blood) surveys (through 1991) are included in the 1994 *Cryptosporidium* Criteria Document.

Table 2. Geographic Distribution of Human Cryptosporidiosis

North America	Caribbean	Central/ South America	Africa	Europe
Canada	Cuba	Argentina	Algeria	Austria
Mexico	Haiti	Brazil	Burundi	Belgium
United States	Jamaica	Colombia	Cameroon	Czechoslovakia
	Puerto Rico	Chile	Ethiopia	Denmark
Pacific	St. Lucia	Costa Rica	Gabon	England
Australia	Tobago	Ecuador	Ghana	Finland
Malaysia	Trinidad	El Salvador	Guinea	France
New Zealand	Virgin Islands	Guatemala	Guinea-Bissau	Germany
Papua-New Guinea		Panama	Ivory Coast	Greece
Philippines	**Asia**	Peru	Kenya	Hungary
Singapore	Bangladesh	Uruguay	Liberia	Ireland
	Belarus	Venezuela	Mauritania	Italy
Middle East	Cambodia		Mauritius	Lithuania
Egypt	China		Morocco	Netherlands
Iran	India		Nigeria	Poland
Israel	Japan		Rwanda	Portugal
Kuwait	Korea		South Africa	Romania
Saudi Arabia	Myanmar Republic		Sudan	Serbia
	Pakistan		Togo	Spain
	Russia		Tunisia	Sweden
	Sri Lanka		Uganda	Switzerland
	Taiwan		Zaire	Turkey
	Thailand		Zambia	Wales
			Zimbabwe	

B. Occurrence in Water

1. Surface Water

Since the 1994 Criteria Document was published, dozens of reports have appeared in the literature further characterizing the occurrence of *Cryptosporidium* in surface waters. Several of these articles adequately summarize this large body of work. Lisle and Rose (1995) reviewed the literature for more than 25 studies involving outbreaks, occurrence, monitoring, and detection, as well as regulatory implications. They 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 oocysts/L. They concluded that better methods were needed for oocyst recovery, detection, and treatment. In another major study, LeChevallier and Norton (1995) reported finding oocysts in 60.2% of surface waters tested in the U.S. and Canada. Three companion articles in the September 1997 issue of the Journal of the American Water Works Association summarized the current state of knowledge of *Cryptosporidium* occurrence in watersheds (Crockett and Haas, 1997), rivers (States *et al.*, 1997) and reservoirs (LeChevallier *et al.*, 1997). All three studies concluded that any surface water is subject to a complex set of watershed characteristics (buffer zones, slope, land use, watershed management, storm water management, sewage treatment practices, sediment types, soil types, vegetation, population density, pathogen sources, best management practices, and recreational uses) and watershed processes (precipitation, snow and ice-melt-derived runoff, sediment resuspension, dumping, spills, wastewater treatment plant failures, temperature fluctuations, and algal blooms) that may lead to the presence of oocysts.

2. Groundwater

According to the 1994 *Cryptosporidium* Criteria Document, oocysts are found less frequently in groundwater than in surface water and consequently very few cryptosporidiosis outbreaks have been traced to groundwater contamination. Kramer *et al.* (1996), in a U.S. national survey for the presence of *Cryptosporidium* oocysts in drinking water, showed that, of the five outbreaks recorded from 1993 to 1994, two outbreaks resulted from untreated well water. Both of the outbreaks occurred in Washington State, one in 1993, which accounted for 7 cases of human cryptosporidiosis, and one in 1994 accounting for 104 cases (Rose *et al.*, 1997). Hancock *et al.* (1998) recently reported a study of 199 groundwater samples tested for *Cryptosporidium.* They found that 5% of vertical wells, 20% of springs, 50% of infiltration galleries, and 45% of horizontal wells tested were positive for *Cryptosporidium* oocysts. This new data will force environmental microbiologists and regulators to reassess previous assumptions that groundwater is inherently free of parasites.

C. Occurrence in Soil

Most *Cryptosporidium* research has centered on detecting oocysts in either water or biological samples. Limited studies have been performed to ascertain the presence or viability of *Cryptosporidium* in soil. Transport of *Cryptosporidium* oocysts to water from fecal-contaminated soil within a watershed during weather events was suggested as the most probable mechanism of source water contamination in several documented outbreaks (Kramer *et al.*, 1996). Mawdsley *et al.* (1996a) reported on the vertical movement of *Cryptosporidium* oocysts through intact, 30-cm soil cores. Transport of oocysts through the soil cores was greater in silty loam and clay loam than in loamy sandy soil, which was exactly the opposite of what the researchers expected. Twenty-one days after inoculation, the majority of oocysts still in the soil remained in the

top 2 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 implied that a large proportion of oocysts are retained in the runoff rather than being adsorbed onto the soil surface. Research funded by the American Water Works Association Research Foundation, aimed at creating a better understanding of watersheds and soil oocyst interactions, is ongoing.

A new method was recently reported by Anguish and Ghiorse (1997) for examining *Cryptosporidium* oocysts in media other than water. They used a computer-assisted laser-scanning microscope equipped for confocal laser scanning and color video microscopy to examine two agricultural soils, barnyard sediment and a calf fecal sample. The authors concluded that this technique provides a better approach for oocyst identification and enumeration, as well as for *in situ* assessment of cellular activity and viability. This technology could have great utility for detecting *Cryptosporidium* in soil.

D. Occurrence in Air

The 1994 *Cryptosporidium* Criteria Document cited no data to show that *Cryptosporidium* is found in ambient air. A review of the current literature indicates that this continues to be an unresearched area.

E. Occurrence in Food and Beverages

The occurrence of several foodborne outbreaks in recent years has 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), and

eight types of fresh, Costa Rica-grown produce (Monge *et al.*, 1995). A foodborne outbreak of cryptosporidiosis associated with eating foods containing uncooked and possibly unwashed green onions in Spokane, Washington has been reported (Quinn *et al.*, 1998). Refrigeration does not compromise oocyst viability. The influence of temperature on oocyst survival is discussed in section III-G. Recent studies evaluating oocyst viability (assessed by vital dye staining) showed greater than 85% of oocysts present in beer and cola made from intentionally contaminated water lost their viability, while only 35% of oocysts in reconstituted infant formula and orange juice lost viability (Friedman *et al.*, 1997). These authors speculated that the decreased pH of the carbonated beverages triggers premature excystation.

F. Specific Disease Outbreaks

1. Outbreaks Associated with Drinking Water

The 1994 *Cryptosporidium* Criteria Document described a number of waterborne disease outbreaks attributed to *Cryptosporidium.* The 1989/1990 and 1991/1992 MMWR papers on waterborne disease outbreaks prepared by the U.S. Centers for Disease Control and Prevention (Herwaldt *et al.*, 1991 and Moore *et al.*, 1993) were not included in the 1994 document. Herwaldt *et al.* (1991) found that no outbreaks in 1989 or 1990 were attributed to *Cryptosporidium.* Moore *et al.* (1993) reported that, for 7 of the 11 outbreaks for which an agent was determined, a protozoal parasite (*Giardia lamblia* or *Cryptosporidium*) was the etiological agent. Since 1994, numerous papers have appeared in the literature that more completely describe the previous outbreaks and document new outbreaks; these are described in the remainder of this section.

Additional research articles have been published specific to the 1993 Milwaukee outbreak. Addiss *et al.* (1996) reported on the effectiveness of point-of-use water treatment devices during the Milwaukee outbreak. They concluded that using sub-micron point-of-use devices may significantly reduce the risk of waterborne cryptosporidiosis. Rodman *et al.* (1997) studied the utility of monitoring sales data on nonprescription antidiarrheal medications to detect enteric disease outbreaks. Although the technique would have been useful in detecting the Milwaukee outbreak, the information from this study showed that the costs incurred in collecting the data and the absence of increased sales of antidiarrheal medications during other outbreaks limit the utility of this type of analysis in other cities. Turbidity spikes at the Milwaukee water treatment plant correlated strongly with hospital visits for gastrointestinal disease prior to 1993 (Morris *et al.*, 1998), indicating that cryptosporidiosis was occurring in Milwaukee for more than a year before the outbreak. Eisenberg *et al.* (1998) confirmed this finding and further concluded that 85% of the outbreak infections could have been avoided if Cryptosporidium had been identified as the etiological agent in the smaller outbreak. Researchers concluded, after studying Milwaukee death certificates from before and after the outbreak, that waterborne outbreaks of cryptosporidiosis can result in significant mortality, particularly among immunocompromised populations (Hoxie *et al.*, 1997). Fox and Lytle (1996) published a summary article outlining the results of the investigation of the Milwaukee outbreak by the USEPA. Moss *et al.* (1998) reported on an outbreak of cryptosporidiosis involving more than half of the crew members of a Coast Guard cutter which had filled its water tanks with water from Milwaukee during March of 1993.

Duke *et al.* (1996) reported an outbreak of cryptosporidiosis in Northumberland, U.K. The source water was a private untreated water supply that appeared to be contaminated by run-off slurry from surrounding fields and/or lamb carcasses found in a collection chamber connected to the water supply. Atherton *et al.* (1995) also

reported an outbreak of cryptosporidiosis in northern England which was attributed to failure of the public drinking water system to remove oocysts. The outbreak onset was characteristic of a point source infection occurring during a period of heavy rainfall at the reservoir. Maguire *et al.* (1995) reported an outbreak in London, U.K., where 44 individuals were confirmed to have *Cryptosporidium* infections acquired from drinking tap water. Bridgman *et al.* (1995) reported 47 cases of cryptosporidiosis linked to two groundwater sources in northwestern England. In this case, it was found that one of the water sources could be contaminated with surface water from a field containing livestock waste in times of heavy rainfall, similar to that experienced during the time of infection.

Leland *et al.* (1993) wrote an article not cited in the 1994 *Cryptosporidium* Criteria Document describing the Jackson County, Oregon outbreak of 1992 from an engineering perspective. In a Florida outbreak, 77% of the counselors and campers at a day camp were infected from a municipal drinking water supply (CDC, 1996c). Goldstein *et al.* (1996) reported an outbreak of cryptosporidiosis in Las Vegas, Nevada, within the Lake Mead watershed area, where 78.2% of the cases occurred in immunocompromised persons and 21.8% of the infected individuals were immunocompetent. The Nevada treatment system used state-of-the-art technologies and chemical treatment. Recognition of the outbreak was attributed to surveillance conducted by the State of Nevada, where cryptosporidiosis is a reportable disease.

A number of review articles concerning *Cryptosporidium* outbreaks have been published since 1994. Rose *et al.* (1997) briefly described a number of outbreaks associated with drinking water, and Solo-Gabriele and Meumeister (1996) presented an overview of U.S. outbreaks, supporting their view that current practices and

regulations are inadequate to protect consumers from waterborne disease. The *MMWR Surveillance for Waterborne-Disease Outbreaks - United States, 1993-1994* (CDC, 1996b) reported that 10 of 29 total waterborne disease outbreaks were associated with the protozoans *Giardia* and *Cryptosporidium.* A national survey over a 2-year test period (1993 and 1994) identified five outbreaks resulting in 403,271 cases involving *Cryptosporidium* oocysts in drinking water (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. All three outbreaks were attributed to surface water as the source. The remaining two outbreaks resulted from contaminated groundwater: one from a private well in Washington State (resulting in seven cases), and the other from a community well, also in Washington State (resulting in 104 cases). Some notable outbreaks in the United States from 1984 to 1995 which were associated with drinking water and the deficiencies which caused them are summarized in Table 3.

In addition to the outbreaks described in the literature, a newsletter called *Cryptosporidium Capsule* provides additional, anecdotal information about suspected outbreaks in drinking water in Devon, U.K. in 1995, drinking water in Maine in 1995, drinking water in Collingwood Ontario in 1996, lakes in Cranbrook and Kelowna, British Columbia, in 1996, and a groundwater outbreak in the U. K. in 1997.

Table 3. Outbreaks of Cryptosporidiosis Associated with Drinking Water in the U.S.

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

NC = Non-community; C = Community; I = Individual

No cases of cryptosporidiosis were reported in the waterborne disease outbreak survey published by the CDC from 1989 to 1990; however, the large number of cases of acute gastrointestinal illness (AGI) of unknown etiology may have included illness caused by *Cryptosporidium.* Of the total number of AGI cases reported in 1989 and 1990, 56% (2,402 of 4,288) were unexplained (Herwaldt *et al.*, 1991). In the U.S. surveillance report from 1991 to 1992 (Moore *et al.*, 1993), approximately 20% of the total enteric illness cases were caused by *Cryptosporidium.* Cases of AGI represented 76.5% of the total enteric illnesses reported from 1991 to 1992, some of which were likely caused by *Cryptosporidium.* These observations suggest that efforts to identify *Cryptosporidium* as the etiological agent during outbreaks frequently fail.

Two articles have been written about the inadequacies of current surveillance practices in detecting and preventing cryptosporidiosis from drinking water. Craun *et al.* (1997) pointed out that the coliform test can no longer be used as the sole indicator of a water's microbiological safety. Frost *et al.* (1996) focused on the importance of epidemiological surveillance and collaboration between water purveyors and community public health departments to enhance public safety.

2. 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 specifically linked to *Cryptosporidium.* Three of the *Cryptosporidium* outbreaks were associated with motel swimming pools; two were associated with community swimming pools. All five pools were filtered or chlorinated; one had a malfunctioning filter. None of the other pools had identifiable treatment deficiencies. The inability of chlorine levels normally used in swimming pools to kill *Cryptosporidium*, coupled with poor pool filtration equipment maintenance practices, have been suggested as the primary cause of swimming pool-related cryptosporidiosis.

Outbreaks associated with recreational waters can be difficult to recognize because the individuals using such facilities may reside in widely separated geographical areas. Forty-four individuals contracted cryptosporidiosis after swimming in a Los Angeles pool where an accidental fecal release occurred (CDC, 1990; Sorvillo *et al.*, 1992). McAnulty *et al.* (1994) reported a community-wide outbreak of cryptosporidiosis in Lane County, Oregon that was linked to swimming at a wave pool with inadequate sand filtration equipment. MacKenzie *et*

al. (1995a) reported on a swimming pool-related outbreak involving 51 individuals. The outbreak occurred in a city 75 miles from Milwaukee about 30 days after the massive Milwaukee outbreak. The authors suggested that increased attention should be given to preventing swimming pool-related outbreaks following outbreaks of cryptosporidiosis associated with water supplies. Cryptosporidiosis associated with two pools in Dane County, Wisconsin was reported during the summer of 1993 (CDC, 1994). Kramer *et al.* (1998) reported an outbreak with 38 infected individuals who contracted cryptosporidiosis while swimming in a recreational lake. They speculated that contamination of the lake came from either infected swimmers or run-off.

3. Foodborne Outbreaks

Foodborne transmission of cryptosporidiosis has only rarely been reported. 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 was the first large outbreak in which foodborne *Cryptosporidium* could be identified and documented as the causative agent (Millard *et. al.*, 1994). A survey, completed for 611 (81%) of the estimated 759 attendees at the fair, found 160 (26%) cases of primary cryptosporidiosis. *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. Two more foodborne outbreaks, one involving apple cider and another associated with green onions, are 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. These outbreaks underscore the need for agricultural producers to take precautions to avoid contamination of foodstuffs by infectious agents commonly present in the farm environment. 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 cryptosporidiosis in 50 attendees of a social gathering who ate a salad contaminated during preparation by a day care worker (CDC, 1996a). Laberge *et al.* (1996) prepared a review article in which they list foods associated with *Cryptosporidium* infections that include unpasteurized milk, sausage, raw beef, kefir, pelleted feed, silage, powdered milk, raw tripe, and apple cider. Casemore *et al.* (1997) referred to sausage, offal, and raw milk contamination by *Cryptosporidium* but did not link these foods to specific outbreaks. Harp *et al.* (1996) reported that standard commercial pasteurization techniques kill 100% of *C. parvum* oocysts. Methods to detect oocysts in food have not been optimized.

4. Outbreaks among Travelers

The 1994 *Cryptosporidium* Criteria Document stated that cryptosporidiosis has emerged as an important cause of traveler's diarrhea and indicated that illnesses frequently occur in travelers visiting developing countries. However, reports since 1994 indicate that travelers within developed countries such as the United States have also acquired *Cryptosporidium* infections.

During the Milwaukee outbreak in 1993, visitors became infected with *Cryptosporidium* as a consequence of drinking water in that city. In addition, upon returning home, they transmitted the parasite to members of their households (MacKenzie *et al.*, 1995b).

5. 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, 2001).

6. Outbreaks Among Sensitive (Immunocompromised) Subpopulations

While outbreaks of cryptosporidiosis are rarely limited to immunocompromised subpopulations, cases in immunocompromised individuals may be detected first because these individuals are more likely to be diagnosed. For example, an outbreak of cryptosporidiosis in Las Vegas, Nevada (Clark County within the Lake Mead watershed area) was first recognized among HIV-infected people (Goldstein *et al.*, 1996; Roefer *et al.*, 1995). Immunocompromised persons accounted for 78.2% of the cases in this outbreak. Although these individuals had an increased risk of dying by the end of the outbreak (compared to immunocompromised individuals without cryptosporidiosis), they did not have an increased one-year mortality rate.

G. Environmental Factors

Because *Cryptosporidium* oocysts are remarkably resistant to inactivation in the environment, the survival of *Cryptosporidium* under a variety of environmental conditions has been evaluated by a number of investigators. While the majority of these studies have considered the effects of physical antagonism (e.g., freezing, heating, UV exposure), studies have also been conducted to consider the role of microbial antagonists (microbial predation), chemical antagonists (such as disinfection) and aging. This section will focus primarily on aspects of physical antagonists in the environment. The aspects of chemical disinfectants are discussed below in

Section VII, Inactivation of *Cryptosporidium* under Water Treatment Practices. Walker *et al.*, (1998) have reviewed laboratory and field studies on the survival and transport of *C. parvum* oocysts.

Robertson *et al*. (1992) evaluated the sensitivity of *C. parvum* oocysts to a variety of environmental pressures such as freezing, dessication, and water treatment processes, as well as in physical environments commonly associated with oocysts. Approximately 97% of the test oocysts were inactivated after 18 days at -22°C, suggesting that the levels of viable oocysts in surface waters subjected to freezing might be influenced by seasonal temperature variations. After 2 hours of drying oocysts at room temperature, only 3% of oocysts were still viable and, after 4 hours, no oocysts were viable. None of the water treatment processes investigated (i.e., alum floccing, liming, and ferric sulfate floccing) had any effect on oocyst viability when pH was corrected. When stored at 4°C, the percentage of oocysts remaining viable in stool samples decreased steadily with time. (In the study, the relationship between oocyst viability and and time varied with individual.) After 176 days in tap water, river water, or cow feces, there was a statistically significant increase in the proportion of dead oocysts in test samples. Seawater was even more lethal to oocysts, with a statistically significant increase in dead oocysts by 35 days of exposure to the test conditions. In summary, the work of Robertson *et al*. (1992) demonstrates that *C. parvum* oocyst viability is sensitive to a wide range of typical environmental conditions while remaining relatively insensitive to some water treatment processes. Their research also emphasizes that oocyst viability is also dependent on the amount of time to which oocysts are exposed to environmental conditions.

Cryopreservation studies conducted by Fayer *et al.* (1991) and Fayer (1997) indicate that oocyst survival depends upon the temperature and duration of freezing conditions, implying that *C. parvum* oocysts are not necessarily rendered noninfectious by being frozen. In another study, Fayer and Nerad (1996) demonstrated that the infectivity of *C. parvum* oocysts after freezing is dependent on the temperature and duration of freezing. In general, shorter freezing times are required to neutralize infectivity when lower freezing temperatures are employed (e.g., 1 hour at -70°C vs. 168 hours at -15°C to completely neutralize infectivity) (Fayer and Nerad, 1996). Temperature stability studies were also conducted by Sattar *et al.* (1999) who evaluated the freeze/thaw susceptibility of various preparations of oocysts, including highly purified preparations as well as infected calf feces. This study indicated that oocyst stability under freezing conditions is at least partially dependent upon the surrounding matrix, with fecal material conferring a cryopreservative effect on oocysts.

In the absence of freezing conditions, colder water temperatures tend to promote the survival of most microorganisms. *C. parvum* may survive outside of mammalian hosts for several months or more depending upon water temperature (Straub *et al.*, 1994). Fayer *et al.* (1998b) investigated the effect of water temperatures ranging from -10°C to 35°C on oocyst infectivity. As water temperature increased to a maximum of 20°C, oocysts remained infectious for longer exposure times. For example, oocysts retained their infectivity for 1 week in -10°C water but remained infectious for up to 24 weeks in 20°C water (Fayer *et al.*, 1998b). As water temperatures increased above 20°C, oocysts retained their infectivity for shorter exposure times (Fayer *et al.*, 1998b).

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. Anderson (1985) evaluated the infectivity of *Cryptosporidium* oocysts following exposure to a variety of moist heat treatments. Warming oocysts to 45°C for 5 to 20 minutes was effective in completely neutralizing their infectivity (Anderson, 1985). The efficiency in reducing the infectivity of *C. parvum* oocysts through exposure to high temperatures for short time periods, such as the conditions used in pasteurization processes, was examined by Harp *et al.* (1996). They 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.

Further research on the effects of dessication on *C. parvum* oocysts demonstrates that typical environmental conditions are effective in reducing infectivity. Anderson (1986) examined the infectivity of oocysts from calf fecal samples which had been subjected to drying in either winter or summer months. In summer temperatures (i.e., 18°C to 29°C) with approximately 60% humidity, oocysts completely lost infectivity in 1 to 4 days. Experiments conducted in winter, with temperatures ranging from -1°C to 10°C and humidity of approximately 60%, resulted in a complete loss of infectivity within 2 to 4 days. Control samples kept moist or kept moist and refrigerated retained infectivity for up to 14 or 21 days, respectively.

Limited studies have been conducted on the effects of physical shear on oocyst viability; these studies have attempted to assess the potentially abrasive effects of oocyst contact with sand and gravel particles or through 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. Sattar *et al.* (1999) conducted studies to evaluate the synergistic effects of mixed sand reactors upon disinfection efficiency with chlorine, and they observed that shear stress enhanced chlorine inactivation. Sattar *et al.* (1999) also evaluated the effects of microbial predation upon oocyst survival and observed that oocysts incubated in dialysis cassettes that were suspended in natural waters exhibited significantly longer survival times when bacterial populations were excluded from the suspension water, implying that microbial predation may play an important role in determining oocyst survival in natural waters.

H. Summary

In summary, cryptosporidiosis is zoonotic, widespread, and often associated with surface water, groundwater, recreational water, and contaminated food and drink. Outbreaks associated with these sources continue to occur among travelers, children in day care centers, and immunocompromised as well as healthy individuals throughout the world. Ten valid species of *Cryptosporidium* have been described in more than 150 mammalian species, 30+ avian species, 57 reptilian species, 9 species of fish, and 2 amphibian species. Until more research is completed, public health workers can do little more than speculate on the human infectivity of all the C*ryptosporidium* species. Currently, there is little information published on the cross-reactivity of the nine species to commercially available antibodies used for detection of the organism. Immunocompromised individuals such as those with HIV infections or AIDS, very young children, the elderly, and individuals undergoing therapeutic treatment for cancer are more likely to acquire an infection, develop cryptosporidiosis, and show more severe clinical symptoms. 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. Infected individuals will shed oocysts in their feces and can transmit

the infection to other family or community members. In addition, day care centers for children, due to their high density of a sensitive population, are a potential source for secondary spread of cryptosporidiosis from infected children to others both within and outside of their households. Research on environmental factors has confirmed previous work showing that oocysts are highly refractory to environmental stressors.

IV. Health Effects in Animals

A. Symptomatology and Clinical Features

The 1994 *Cryptosporidium* Criteria Document included general information on the symptomatology and clinical features of cryptosporidiosis in a limited number of domesticated mammals such as calves and lambs. Cryptosporidiosis has also been documented in many additional mammals, both domestic and wild, as well as several species of birds, reptiles, and fish (see section III-A). Two recent review articles contain comprehensive information on the symptomatology and clinical features of cryptosporidiosis in these different groups of animals (Fayer, 1997; O'Donoghue, 1995).

In general, the development of cryptosporidiosis depends on the species, age, and immune status of the host (Fayer, 1997). Younger animals and animals with less developed or compromised immune systems are generally more susceptible to severe infection than healthy adult animals. In many cases, healthy adult animals that become infected are asymptomatic or exhibit only mild clinical signs (O'Donoghue, 1995). A summary of the symptomatology and primary clinical features of infected animals follows.

Mammals: Most clinical cases of cryptosporidiosis in mammals involve infection by *C. parvum.* The most common feature of cryptosporidiosis in mammals is profuse, watery diarrhea that may be pale yellow in color and may have a distinct offensive smell. Other clinical signs of infection include dehydration, fever, anorexia, weight loss, weakness, and progressive loss of condition (O'Donoghue, 1995). Most animals will recover spontaneously within 1-2 weeks of infection. Histopathological observations may reveal several lesions in the small intestine, including mild to moderate villous atrophy and loss of epithelial cells. Developmental stages of the parasite are often seen within the small intestine and occasionally elsewhere (stomach, colon, liver, lungs) (O'Donoghue, 1995). Fayer (1997) provided detailed descriptions of the symptomatology and clinical features of cryptosporidiosis in several species of ruminant and non-ruminant mammals.

Birds: Two *Cryptosporidium* species, *C. meleagridis* and *C. baileyi*, are known to cause infection in birds. Avian cryptosporidiosis appears as either a respiratory, enteric, or renal disease (Fayer, 1997; O'Donoghue, 1995). Normally, only one condition manifests during an outbreak, and respiratory infections are more common than enteric or renal infections (Fayer, 1997; O'Donoghue, 1995). Clinical signs of respiratory infections include rales, coughing, convulsive sneezing, and dyspnea (O'Donoghue, 1995). Excess mucus may exist in the trachea, sinuses, and nasal passages, and fluid may be present in the air sac (Fayer, 1997). Histopathological changes may include hypertrophy and hyperplasia of the respiratory epithelium, with reduced or absent ciliation (O'Donoghue, 1995). Parasites may be detected throughout the respiratory tract including the nasopharynx, larynx, trachea, and bronchi (O'Donoghue, 1995).

Clinical signs associated with enteric infections caused by *Cryptosporidium* in birds include mild to severe diarrhea, dehydration, malaise, weight loss, and weakness. Histopathology may reveal atrophy and fusion of villi, along with epithelial hyperplasia and hypertrophy, as well as other malformations (O'Donoghue, 1995). Parasites are found primarily in the gastrointestinal tract (O'Donoghue, 1995).

Renal infections in birds have been detected only at necropsy (O'Donoghue, 1995; Fayer, 1997). In these cases, the kidneys were pale in color (Fayer, 1997) and enlarged (O'Donoghue, 1995). Hypertrophic and hyperplastic epithelial cells were detected throughout the kidney (O'Donoghue, 1995; Fayer, 1997). Parasites were observed in the collecting ducts and convoluted tubules (O'Donoghue, 1995).

Reptiles: *C. serpentis* is the only valid, named species of *Cryptosporidium* causing infection in reptiles, although up to five species may exist based on oocyst morphology (Fayer, 1997). Most reports of infections in reptiles have involved clinical or subclinical infections in captive reptiles (especially snakes); only subclinical infections have been reported in wild reptiles (Fayer, 1997). Cryptosporidiosis in snakes is characterized by anorexia, regurgitation, lethargy, firm midbody swelling, weight loss, and death (Fayer, 1997). Interestingly, most infections in snakes have been detected in mature animals. In addition, most infections have been associated with chronic gastric disease, as opposed to the acute enteritis which is common in mammals with cryptosporidiosis. Infections and intermittent oocyst shedding in snakes may last for several months to 2 years (O'Donoghue, 1995). Histopathological observations have included inflammation, hyperplasia, and hypertrophy of the gastric glands (O'Donoghue, 1995).

Clinical features of cryptosporidiosis reported in lizards have included primarily subclinical gastric infections, while cryptosporidiosis in tortoises involves gastritis and regurgitation, or progressive wasting (O'Donoghue, 1995).

Fish: Except for the original report of cryptosporidiosis in fish (Hoover *et al*., 1981), infections in fish have not been associated with clinical symptoms. The original report described a progressive illness in a tropical marine fish (*Naso lituratus*) which was characterized by anorexia, emaciation, regurgitation, and passage of feces containing undigested food. Although developmental stages of the parasite were found attached to the intestinal mucosa, no pathogenic changes were evident in this fish. Since then, parasites have been detected in the intestines or stomach of other fish, but few histopathological changes have been described (O'Donoghue, 1995).

B. Therapy

Treatment of cryptosporidiosis in animals involves a combination of prophylactic and chemotherapuetic drugs along with other preventative measures. As stated in the 1994 *Cryptosporidium* Criteria Document, there is no approved effective treatment for cryptosporidiosis in animals. However, numerous drugs have been tested in studies that focused both on the treatment of naturally acquired infections and the treatment or prophylaxis of experimentally induced infections in animals. The majority of efficacy evaluations of agents tested in animals have involved prophylactic as opposed to therapeutic drug regimens (Blagburn and Soave, 1997).

A recent review article lists the numerous anticryptosporidial drugs that have been evaluated in animals (Blagburn and Soave, 1997). The findings of the review article are summarized below.

- Most studies have been conducted in laboratory rodents, including mice, rats, and hamsters. Over 30 compounds have proven effective against cryptosporidiosis in rodents, including maduramicin, alborixin, lasalocid, and salinomycin. In some cases, efficacies of the drugs tested exceeded 90% compared to control (nontreated) animals.

- Several anticryptosporidial drugs have also been tested in ruminants. Among the drugs demonstrating activity against *C. parvum* infections are paromomycin, lasalocid, halofuginone, and sulfaquinoxaline.

- Anticryptosporidial drugs have also been tested in several other types of animals including several species of birds and reptiles, as well as mammals (pigs and cats). There has been little success in identifying successful drugs in these animals, although some success was achieved in treating cryptosporidiosis in snakes.

Prevention of cryptosporidiosis in animals is best achieved by eliminating contact with viable oocysts. This is particularly difficult in settings with large numbers of animals such as farms or zoos (Blagburn and Soave, 1997). To prevent infections in these types of settings, infected animals should be quarantined in facilities that can be cleaned and disinfected, contaminated articles and the clothing of animal care workers should be cleaned thoroughly or discarded, clean food and water should be provided, and access of rodents and other wild animals should be restricted (Blagburn and Soave, 1997). Prevention can also be enhanced by ensuring that neonatal mammals receive adequate amounts of colostrum early in life (Blagburn and Soave, 1997).

Treatment of animals suffering from cryptosporidiosis is similar to that in humans, namely, rehydration with fluids and electrolytes along with antidiarrheal drugs (Blagburn and Soave, 1997). In addition, chemotherapy with anticryptosporidial drugs may be initiated.

C. Epidemiological Data

The majority of epidemiological data for cryptosporidiosis in animals is confined to economically important livestock, especially ruminants (Casemore *et al.*, 1997). Three extensive reviews exist which describe the epidemiological data available for animals, including both domestic and wild animals (Casemore *et al.*, 1997; Fayer, 1997; O'Donoghue, 1995). These reviews include information on the prevalence and spread of cryptosporidiosis in many groups of animals including cattle, sheep, goats, pigs, horses, dogs, cats, deer, mice, and several other small mammals, as well as many species of birds, reptiles, and fish.

In general, clinical infections are seen primarily in neonates and immunocompromised animals. Age-related resistance has been documented in several species, and the age of an animal upon infection can greatly alter the severity of the infection and the prepatent period (Casemore *et al.*, 1997). Adult animals often appear asymptomatic even when shedding small numbers of oocysts (Casemore *et al.*, 1997; Fayer, 1997; O'Donoghue, 1995). Serologic surveys in animals suggest much higher prevalence rates of cryptosporidiosis, especially in adults (Casemore *et al.*, 1997; Fayer, 1997). This high prevalence may be due to cross-reaction of animal sera infected with coccidial parasites rather than from actual infections (Casemore *et al.*, 1997).

Potential sources of infection in animals include other infected animals of the same or different species (i.e., 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).

Additional epidemiological studies reported in the literature, but not mentioned in the review articles, describe the prevalence of cryptosporidiosis in the following animals:

cattle/calves (Fayer *et al.*, 1998a; Olson *et al.*, 1997; Perez *et al.*, 1998; Pena *et al.*, 1997)
horses (Scholes *et al.*, 1998; Bray *et al.*, 1998; Forde *et al.*, 1998; Johnson *et al.*, 1997)
lambs (Bukhari and Smith, 1997)
goats (Koudela and Jiri, 1997; Goyena *et al.*, 1997)
nonhuman primates (Muriuki *et al.*, 1997; Majewska *et al.*, 1997)
rodents (Bajer *et al.*, 1997; Bull *et al.*, 1998)
chickens (Sreter *et al.*, 1996)
ostriches (Jardine and Verwoerd, 1997)
pigeons (Rodriguez *et al.*, 1997)
catfish (Muench and White, 1997)
muskrat (Petri *et al.*, 1997),
African hedgehog (Graczyk *et al.*, 1998a)
dugong (Hill *et al.*, 1997)
deer (Majewska *et al.*, 1997)
slow loris (Majewska *et al.*, 1997)
white rhinoceros (Majewska *et al.*, 1997)
Indian elephant (Majewska *et al.*, 1997)
iguana (Fitzgerald *et al.*, 1998)

D. Summary

Cryptosporidium infections have been documented in many different species of mammals as well as in several species of birds, reptiles, and fish. In general, the severity of the infection depends on the species, age, and immune status of the host. Clinical infections are primarily seen in younger animals and animals with compromised immune systems, while infected healthy adult animals may be asymptomatic or exhibit only mild clinical signs.

Most clinical cases of cryptosporidiosis in mammals involve infections by *C. parvum.* The most common features of cryptosporidiosis in mammals are profuse, watery diarrhea, dehydration, fever, anorexia, and weight loss. Two species of *Cryptosporidium, C. meleagridis* and *C. baileyi*, are known to cause infections in birds. Cryptosporidiosis in birds is characterized by respiratory, enteric, or renal infections. Respiratory infections, which cause rales, coughing, convulsive sneezing, and diarrhea, and enteric infections, which cause

dehydration, weight loss, and weakness, are the most common. The majority of cryptosporidiosis infections in reptiles have been reported in captive snakes. These infections, caused by *C. serpentis*, are characterized by anorexia, postprandial regurgitation, lethargy, and midbody swelling. The only clinical infection described in fish was caused by *C. nasorum* and was characterized by anorexia, emaciation, regurgitation, and passage of feces with undigested food.

Treatment of cryptosporidiosis in animals involves a combination of prophylactic and therapuetic drugs along with other preventative measures. Although there is no approved, effective treatment for cryptosporidiosis in animals, several drugs have been tested in rodent and bovine models and have shown substantial success. Several drugs have also been tested in reptiles and birds with limited success. Prevention of cryptosporidiosis in 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.

The majority of epidemiological data for cryptosporidiosis in animals is confined to economically important livestock, especially cattle. There is also information available on sheep, goats, pigs, horses, dogs, cats, deer, mice, and several other small mammals. These studies show that the age of an animal can greatly alter the severity of the infection and the prepatent period and that the primary sources of infection for animals are other infected animals, mechanical carriers, contaminated feed and water, and other contaminated objects.

V. Health Effects in Humans

A. Symptomatology and Clinical Features

The clinical manifestations of cryptosporidiosis in humans are directly related to the immunocompetence of the host and may include profuse, nonbloody, watery diarrhea that generally resolves spontaneously within 48 hours; however, variability in clinical symptoms is appreciable. Diarrheal symptoms are generally not distinguishable from those caused by other common enteric pathogens. Other symptoms reported by individuals afflicted with cryptosporidiosis include abdominal cramps, vomiting, lethargy, and general malaise. Diarrhea results from a combination of enterocyte damage and physical blockage of the intestinal villi, leading to a disruption in the normal balance of intestinal absorption and secretion (Clark and Sears, 1996). The incubation period in humans is estimated to vary between 2 and 10 days (Arrowood, 1997), with a mean incubation of approximately 7-9 days (Juranek, 1998).

Human volunteer studies have been conducted to assess the infectivity and dose-response of *C. parvum* in humans (DuPont *et al.*, 1995). Sixty-two percent of subjects who ingested doses of *Cryptosporidium* ranging from 30 oocysts to 1 million oocysts acquired infection. The infectious dose causing disease in 50% of the population (ID_{50}) for the Iowa strain of *C. parvum* was 132 oocysts in humans, compared with an ID_{50} of 60 oocysts in neonatal mice; however, the test strain of *C. parvum* in this case was adapted to a mouse model prior to challenge studies, which may account for the disparity in ID_{50} values. The mean and median incubation periods for cryptosporidiosis in the study were 9.0 and 6.5 days, respectively. Infected humans developed clinical enteric symptoms that were associated with excretion of oocysts, although one of the 11 subjects who did not pass oocysts passed a single soft stool on day 10 and exhibited enteric symptoms on days 23 through 31. Symptoms of clinical illness included abdominal pains, cramps, and diarrhea in six subjects; six had nausea; one

reported vomiting; and one had moderate dehydration. Table 4 summarizes the dosing data and related infection rates from the study. Note that volunteers exhibiting enteric symptoms (e.g., diarrhea, loss of appetite) did not test positive for cryptosporidiosis in all cases.

Table 4. Rate of Infection and Enteric Symptoms as a Function of Intended Dosage*

Intended Dose	No. Subjects	No. (%) Infected	No. (%) with Enteric Symptoms	No. (%) with Cryptosporidiosis
30	5	1 (20)	0	0
100	8	3 (37.5)	3 (37.5)	3 (37.5)
300	3	2 (66.7)	0	0
500	6	5 (83.3)	3 (50)	2 (33.3)
1000†	7	7 (100)	5 (71.4)	2 (28.6)
Total	29	18	11	7

* Linear regression analysis of the data yielded an r^2 of 0.983 and an ID_{50} of 132 oocysts.

† The intended dose was 1000 oocysts in two subjects, 10,000 in three, 100,000 in one, and 1 million in one.

Source: DuPont *et al.* (1995)

Follow-up studies indicate that the number of excreted oocysts and the pattern and duration of shedding can vary widely among immunocompetent individuals (Chappell *et al.*, 1996). In the volunteer challenge study, high variability in shedding patterns was observed, and oocysts were observed intermittently in consecutive stool samples, implying that production of oocysts is not uniform and may be influenced by unknown factors. These data may in part account for the observation that fewer than half of the individuals who acquire illness during an epidemic produce stools positive for *Cryptosporidium* when single samples are submitted for diagnostic analysis.

B. Epidemiological Data

Although only 13 cases of cryptosporidiosis had been documented by the CDC in 1982, human cryptosporidiosis has been reported in almost 100 countries spanning the globe since that time (Ungar, 1990). Because *C. parvum* is ubiquitous, infects most mammals, and is highly infectious, all human populations are at risk to some degree (Griffiths, 1998). Screening of select human populations was initiated in the United States during the 1980s, with special emphasis on children and immune-suppressed individuals. However, determining the true prevalence of cryptosporidiosis has proven challenging due to the facts that diagnostic methods have limited sensitivity and the majority of individuals who experience mild to moderate diarrheal illness do not generally seek the services of a physician (Juranek, 1998).

In 1994, a workshop was organized by the National Center for Infectious Diseases (NCID) and the USEPA to assist the Centers for Disease Control and Prevention and state public health departments in providing guidance on public health issues relating to waterborne cryptosporidiosis (Juranek *et al.*, 1995; Addiss *et al.*, 1995). The workshop, titled "Prevention and Control of Waterborne Cryptosporidiosis: An Emerging Public Health Threat," addressed the following topics: surveillance systems and epidemiological study designs, public health responses, cryptosporidiosis in immunocompromised individuals, water sampling methods, and interpretation of results. The recommended approaches to surveillance included the following:

- Making cryptosporidiosis incidents or outbreaks reportable to CDC
- Monitoring sales of antidiarrheal medication through local pharmacies (also described by Rodman *et al.*, 1997)
- Monitoring logs maintained by health maintenance organizations (HMOs) and hospitals for complaints of diarrheal illness

- Monitoring the incidence of diarrhea in nursing homes (also described by Proctor *et al*., 1998)
- Monitoring laboratory data for *Cryptosporidium* (also described by Proctor *et al*., 1998)
- Evaluating water distribution system design in selected cities
- Providing prompt epidemiological assistance during outbreaks

A cohort approach was recommended to facilitate the epidemiological study of outbreak data, with blood tests performed quickly in order to screen out negative subjects. A cohort analysis would demonstrate whether the exposure(s) was associated with subsequent infection or disease. Also, since such research requires large sample sizes, the inclusion of blood tests would make this approach feasible. Strategies recommended for the improvement of public health responses included the identification of methods for rapid notification of the risks for waterborne cryptosporidiosis to agencies, advocacy groups, and the public.

The workshop participants emphasized that boiled water advisories are not essential in the absence of supporting epidemiological information suggesting increases in diarrheal disease in the community. On the subject of cryptosporidiosis in immunocompromised individuals, the workshop concluded that immunocompromised individuals are no more likely than immunocompetent individuals to acquire cryptosporidiosis in an outbreak. However, AIDS patients, patients receiving treatment for cancer, recipients of organ or bone marrow transplants, and individuals who have congenital immunodeficiencies are at greater risk than immunocompetent individuals for developing severe, life-threatening cryptosporidiosis if they become infected.

Additional information on the epidemiologic aspects of cryptosporidiosis in humans is provided in a review by Casemore (1990). The distribution of *Cryptosporidium* in humans from several countries was broken down by

age group, non-human reservoirs, and routes of transmission. This study found that when testing was performed for suspected infections, 60% of the positive findings occurred in children and 30% occurred in adults less than 45 years old. The disease occurred in single sporadic cases, small cluster cases, and short clinical series. *Cryptosporidium* is the third or fourth most commonly identified pathogen in the world, and the reported rates are higher in underdeveloped countries, especially in children (Casemore, 1990). Seasonal and temporal trends vary from country to country and occurrence may indirectly reflect rainfall and farming events such as lambing.

Immunocompromised populations are also at high risk of infection and disease from drinking water contaminated with *Cryptosporidium* oocysts. Clayton *et al.* (1994) studied 41 patients with AIDS who had become infected with *Cryptosporidium*, and they found two significant patterns among these individuals. In 61% of these patients, *Cryptosporidium* was present in the proximal small bowel (i.e., upper small intestine) and the patients had severe clinical disease characterized by malabsorption of nutrients. In the remaining 39%, who had less severe disease, *Cryptosporidium* was seen only in the colon or the stool. An increased susceptibility to cryptosporidiosis in immunocompromised individuals was reported in Kenya, East Africa, where 42 to 44% of the HIV-positive patients at the Kenyatta National Hospital tested positive for *Cryptosporidium*, whereas only 8.6% of the non-AIDS patients carried this pathogen. In Zambia, 25.4% of the HIV-positive patients who were symptomatic carried oocysts and had increased specific anti-*Cryptosporidium* IgG and IgA antibodies, but there was no increase in specific IgM antibodies (Cevallos *et al.*, 1995). This antibody profile suggests that the infection was of prolonged duration.

The cryptosporidiosis outbreak in Las Vegas, Nevada (Roefer *et al.*, 1995) was recognized primarily because of the high incidence among the immunocompromised population and because of state requirements for reporting of disease from this pathogen. Although the water supply in this city was processed by a state-of-the-art system, the protection it provided proved inadequate for immunocompromised persons. Research shows HIV-infected patients who have cleared oocyst infections have much higher levels of specific secretory IgA levels than AIDS patients with chronic cryptosporidiosis (Flanigan, 1994). This indicates either that secretory IgA is involved in recovery from infection, or that it is the only marker for an effective immune response at the mucosal surface.

In a study on asymptomatic carriage of intestinal *Cryptosporidium* by immunocompetent and immunodeficient children, the percentage of carriers among immunodeficient children was 22% compared to 6.4% among immunocompetent children (Pettoello-Mantovani *et al.*, 1995). However, the percentages of symptomatic children in these groups were similar, with 4.4% of the immunocompetent children and 4.8% of the immunodeficient children positive for oocysts.

Greenberg *et al.* (1996) reported on stool sampling from AIDS patients receiving chemotherapy and/or radiotherapy. The study objective was to determine the yield of *Cryptosporidium* oocysts in stools versus biopsies taken from the upper and lower intestines of these patients. Only 53% of 106 patients were positive for oocysts when a single stool sample was taken, but detection increased to 73% positive when multiple stool samples were taken (3.3±0.3, mean±SEM). When sampling was by terminal ileum biopsy, the number of positive samples increased to 91%. From these studies, it appears oocysts may invade the small intestine in immunocompromised individuals with no oocysts detectable in stool examinations. Thus, stool sampling can

be expected to miss a substantial number of *Cryptosporidium* infections in immunocompromised and AIDS patients.

In addition to gastrointestinal disease, AIDS patients can have other complications from *Cryptosporidium* infection such as respiratory cryptosporidiosis (Mifsud *et al.*, 1994). Clavel *et al.* (1996a) reported cases of intestinal cryptosporidiosis with pulmonary involvement in AIDS patients who had diarrhea and were positive for *Cryptosporidium* oocysts.

Patients with cancer may be immunocompromised as a side effect of their therapeutic treatment. Therefore, these patients are likely to have an increased susceptibility to cryptosporidiosis. Tanyuksel *et al.* (1995) examined 106 cancer patients, all of whom were receiving chemotherapy and/or radiotherapy and surgery, and found that 17% of the patients who had diarrhea were positive for *Cryptosporidium* oocysts. They concluded that individuals who are compromised by such treatments are at high risk for *Cryptosporidium* infections.

Logar *et al.* (1996) evaluated the occurrence of *C. parvum* in Slovenia and reported a higher incidence of cryptosporidiosis in older patients and young children. The authors believed that the infections in older patients and young children (median age 3 years) were due to lowered resistance or immune response. In the older patients, *Cryptosporidium* infections appeared to be a consequence of other diseases or secondary to irradiation or other immunocompromising treatments common in this age group. Considerable evidence exists to show that immunological deficiency is a natural consequence of aging (Miller, 1996). Casemore (1990) observed that the severity of disease from infection is greatest among children under 5 years of age and among

immunocompromised patients (e.g., AIDS or cancer patients), and that the impact is greatest in developing countries.

The 1994 *Cryptosporidium* Criteria Document discussed the high prevalence of cryptosporidiosis in children and notes that the evidence comes primarily from reports of diarrhea in day care centers. Cryptosporidiosis is now recognized as a significant disease in childcare settings (Cordell and Addiss, 1994). Additional data on the effectiveness of prevention and control strategies, as well as on the economic impact of these outbreaks on the community, state, and country, should be collected. There is evidence that cryptosporidiosis affects children in other countries. In China, 42 to 58% of a cross-section of children less than 16 years of age were serologically positive for *Cryptosporidium* (Zu *et al.* 1994). Among a similar group in Virginia, less than 17% tested positive. The data from the review by Cordell and Addiss (1994) indicate that in impoverished communities, this parasitic infection is highly endemic and occurs in early childhood. Single stool samples from 1,000 apparently healthy children (ages 6 through 14) in Jordan showed that 4% of the children had oocysts and, among these children, 37% were symptomatic (Nimri and Batchoun, 1994). Brandonisio *et al.* (1996) reported 7 (1.9%) of 368 children hospitalized (for unspecified reasons) in Italy tested positive for oocysts (359 of these children were immunocompetent and 9 were HIV infected). Six of the seven cases were in immunocompromised children. Brannan *et al.* (1996) found that 12% of the children in Romania were carrying *C. parvum* oocysts, although 73% of these children had either IgA or IgG antibodies to this protozoal parasite.

Adegbola *et al.* (1994) reported that the occurrence of *Cryptosporidium* infection in Gambian children has seasonal peaks associated with rain and high relative humidity. Factors accounting for the seasonal distribution

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 the impact of the rainy season on domestic and environmental hygiene. For additional information regarding cryptosporidiosis in children, refer to "*Cryptosporidium*: Risk for Infants and Children" (USEPA, 2001).

C. Treatment: Clinical Laboratory Findings and Therapeutic Management

Cryptosporidiosis is self-limiting in most immunocompetent patients as well as in many immunocompromised patients. The recommended management of *Cryptosporidium*-infected patients includes careful monitoring of hydration and electrolyte balance, with oral or intravenous hydration and nutrition as necessary. Antimotility agents (i.e., opiates or somatostatin and its analogues) may be helpful to prevent 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 (Griffiths, 1998).

In patients infected with HIV, cryptosporidiosis has been a major cause of morbidity and mortality, resulting from dehydration and malnutrition (Blanshard *et al.*, 1997). Since the publication of the 1994 *Cryptosporidium* Criteria Document, several new treatment strategies have been pursued. The most promising development is associated with the introduction in 1996 of protease inhibitors for the treatment of HIV infection. Le Moing *et al.* (1998) examined data on the prevalence of intestinal cryptosporidiosis in HIV-infected patients for the period from January, 1995, to December, 1996. They observed a decrease in the prevalence of cryptosporidiosis at the same time that protease inhibitors first gained widespread use in this population. Although this finding

does not prove that protease inhibitors were responsible for the decrease in cryptosporidiosis, the study authors noted that there was no new treatment for cryptosporidiosis during that time and no change in the number of HIV-infected patients. The study results confirm that protease inhibitors had a beneficial effect on cryptosporidiosis at the population level.

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. Miao *et al.* (1999) examined the effect of three different combinations of antiretroviral therapy with protease inhibitors. After six months of treatment, two of three patients had fecal smears that were negative for *C. parvum*. The third patient stopped treatment after one month due to adverse side effects. While on the treatment, this patient's fecal smears were also negative for *C. parvum* but relapsed within two months after going off antiretroviral therapy. The results suggest that the treatment regimen suppresses *C. parvum* infection when taken for one month but completely eliminates infection after six months of treatment. Maggi *et al.* (2000) conducted a retrospective cohort study to compare the response of HIV-infected patients with cryptosporidiosis to antimicrobial treatment alone or in conjunction with antiretroviral treatment (up to three drugs). The therapeutic effect of antimicrobial treatment and combination antiretroviral therapy (either two or three drugs) on cryptosporidiosis was excellent and was sustained after a lengthy follow-up period of nearly two years. The study authors speculated that the patients' responsiveness to combination antiretroviral treatment was due primarily to an increase in $CD4^+$ cell count rather than decreased viral load. Antimicrobial treatment alone or in conjunction with a single antiretroviral drug was not effective in treating cryptosporidiosis in HIV-infected patients.

To date, no chemotherapeutic agents have been consistently effective in the management of cryptosporidial infections (O'Donoghue, 1995; Blagburn and Soave, 1997). 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).

Spiramycin, a macrolide antibiotic, was described in the 1994 *Cryptosporidium* Criteria Document as showing limited success in the treatment of cryptosporidiosis. Other macrolides that have been evaluated include erythromycin (Connelly *et al.*, 1988), clarithromycin (Jordan 1996), and azithromycin (Vargas *et al.*, 1993; DuPont *et al.*, 1996; Hicks *et al.*, 1996). Spiramycin and erythromycin have shown unacceptable side effects (Connelly *et al.*, 1988). Azithromycin was reported as successful in treating several cases of cryptosporidiosis in HIV-infected patients (DuPont *et al.*, 1996, and Hicks *et al.*, 1996) and cancer patients undergoing chemotherapy (Vargas *et al.*, 1993); however, in a pilot-scale clinical trial with azithromycin (500 mg daily), the compound was ineffective for treating cryptosporidiosis in AIDS patients (Blanshard *et al.*, 1997). Clarithromycin prophylaxis was considered successful in preventing *Cryptosporidium* enteritis based on two retrospective analyses (Jordan, 1996). In the first, a retrospective analysis of 136 AIDS patients revealed that none of the 63 that received clarithromycin 500 mg twice daily developed *Cryptosporidium* enteritis, whereas four patients in the control group developed *Cryptosporidium* enteritis. In the second, none of 217 AIDS patients receiving clarithromycin 500 mg twice daily developed *Cryptosporidium* enteritis over a two-year period. No other studies on clarithromycin were located.

A one-month course of paromomycin led to the remission of 18 of 24 patients with intestinal cryptosporidiosis but 10 patients relapsed once treatment was reduced or stopped (Bissuel *et al.*, 1994). In a pilot-scale clinical trial of AIDS patients with cryptosporidiosis, 60% treated with paromomycin had a complete resolution of diarrhea and a further 5% had some resolution of symptoms, but paromomycin treatment did not eliminate the *Cryptosporidium* infection (Blanshard *et al.*, 1997). A pilot-scale clinical trial of letrazuril treatment for AIDS patients with cryptosporidiosis resulted in an improvement of symptoms in 40% of the treated patients, and 70% stopped excreting cryptosporidial oocysts, but biopsies remained positive for *Cryptosporidium* (Blanshard *et al.*, 1997).

Ionophores with anticoccidial properties have also been evaluated for treatment of cryptosporidiosis in animals (Mead *et al.*, 1995). The most promising agents, maduramycin and alborixin, resulted in 96% and 71% reductions, respectively, in oocysts in immunodeficient mice. Toxicity also was observed in the therapeutic trials with these compounds in mice, and they are considered to be too toxic for human use in their current formulations.

The use of hyperimmune bovine colostrum for the treatment of cryptosporidiosis was described by Crabb (1998). However, considerable variation has been noted in the efficacy of different colostrum preparations (O'Donoghue, 1995).

D. Mechanism of Action

The 1994 *Cryptosporidium* Criteria Document reported that the pathogenic mechanisms of *Cryptosporidium* in cryptosporidiosis are not known. While the pathogenesis remains unclear, more recent work has helped elucidate the process. *Cryptosporidium* sporozoites and merozoites invade the absorptive cells covering small intestinal villi, damaging and eventually killing enterocytes. Forney *et al.* (1996) suggested proteolytic activity is involved in the infectivity of *C. parvum*, based on the interaction between human alpha-l-antitrypsin (ATT) and parasite subcellular components. This suggests that the use of serine protease inhibitors may be useful as a therapeutic strategy. Riggs *et al.* (1996) identified antigens that may have a critical role in sporozoite infectivity and therefore may be suitable molecular targets for passive or active immunization against cryptosporidiosis.

Diarrhea occurs when intestinal absorption is impaired or secretion is increased. 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 NaCl absorption (Clark and Sears, 1996). This disruption in the absorption/secretion balance can lead to diarrhea. Clinical studies in *C. parvum*-infected piglets (Argenzio *et al.*, 1993) have suggested that *Cryptosporidium*-induced diarrhea is of a secretory nature. However, Kelly *et al.* (1996) conducted perfusion studies to measure water and electrolyte transport *in vivo* in five HIV-cryptosporidiosis patients and nine healthy volunteers. There were no differences in net water, sodium, and chloride movement in the jejunum of the two groups. In addition, there was no evidence demonstrating that cryptosporidial diarrhea was due to a secretory state in the proximal small intestine. Other studies have suggested that *Cryptosporidium*-induced diarrhea may be caused by a toxin (Guarino *et al.*, 1994;

Guarino *et al.*, 1995). A thorough review of cryptosporidial pathogenesis can be found in Clark and Sears (1996).

E. Immunity

The importance of cellular immunity in resolving *Cryptosporidium* infection is highlighted by the contrasting ability of immunocompetent and immunocompromised individuals to resolve infections. While depletion of $CD8^+$ cells (Ungar, 1990), NK cells (Rasmussen and Healy, 1992), mast cells (Harp and Moon, 1991), tumor necrosis factor (McDonald *et al.*, 1992), or interleukin-2 (Ungar *et al.*, 1991) did not result in enhanced infection in mice, removal of $CD4^+$ cells and/or gamma interferon caused severe chronic infections (Ungar *et al.*, 1991). Additionally, *Cryptosporidium*-infected immunodeficient mice that were reconstituted with spleen or lymph node cells from immunocompetent Balb/c mice were able to recover from infection, but upon depletion of the $CD4^+$ T cells from the donor, the curative effects were abrogated (Kuhls *et al.*, 1996). In humans, HIV-infected patients with $CD4^+$ counts of 180 cells/mm^3 cleared the infection in 4 weeks, while of those with lower counts, 87% developed chronic disease (Flanigan *et al.*, 1992). Little is known about the gut mucosal response to the parasite. Wyatt *et al.* (1996) used a bovine animal model to examine mucosal immunity during cryptosporidiosis and showed that ileal intraepithelial T lymphocytes are activated coincident with enteric disease. This suggests the importance of cell-mediated activity during *Cryptosporidium* infection.

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). The presence of local and secretory antibodies has also been confirmed (Laxer *et al.*, 1990); however, the role of these antibodies in

combating infection is unclear (O'Donoghue, 1995). Kapel *et al.* (1993) used a time-resolved immunofluorometric assay to determine the presence of *Cryptosporidium* antibodies in 12 HIV-*Cryptosporidium*-infected patients. These patients displayed marked elevation in anti-*Cryptosporidium* IgA and IgM antibody titers. These high antibody titers were not correlated with the gravity of infection in terms of oocyst shedding. Also, there was no evidence of protection even though there was a mucosal immune response. Studies comparing *C. parvum* infections in B cell-depleted mice showed that infections were similar to those in normal mice (Taghi-Kilani *et al.*, 1990).

There is evidence for protective immunity to cryptosporidial infection. Repeat infections in dairy cattle workers occur but are generally much milder than the first infection (Reese *et al.*, 1982). Permanent residents in areas where cryptospordiosis is common often acquire mild or asymptomatic infections; however, 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 approximately 500 oocysts, 1 year following primary infection. Fewer subjects shed oocysts after the second exposure (16% vs. 63%). Although the percentage of subjects with diarrhea was similar, the clinical severity of infection, as determined by the number of unformed stools passed, was less following rechallenge compared to the primary challenge response. The number of IgG and IgA seroconversions increased, but the antibody response did not correlate to the presence or absence of infection.

F. Chronic Conditions

Duration of illness in cryptosporidiosis patients is influenced primarily by the immune response of the individual, with most immunocompetent individuals overcoming the acute enteritis stage within two weeks. Immunocompromised individuals generally present with chronic enteritis which may last as long as the immune impairment. Immunocompromised populations include patients undergoing chemotherapy for treatment of neoplasms, persons undergoing immune suppression treatment to prevent rejection of skin or organ transplants, malnourished individuals, patients who present with concurrent infectious diseases such as measles, the elderly, and AIDS patients. Chronic illness may manifest itself as a series of intermittent episodes or may be persistent. A functional threshold has been established using $CD4^+$ cells to define the probability that infection will resolve; patients presenting with $CD4^+$ counts exceeding 200/ L can generally expect to clear the infection, while those with $CD4^+$ counts falling below this level may suffer chronic infection (Fayer *et al.*, 1997a).

G. Summary

The primary symptom of cryptosporidiosis in humans is fulminant watery diarrhea. The limited data available from human volunteer feeding studies indicate a mean ID_{50} of 132 oocysts. Recent research suggests that the pathological response to *Cryptosporidium* is initiated when the sporozoites and merozoites invade and kill the intestinal epithelial cells (enterocytes). The enterocytes are extruded from the intestinal epithelium, triggering epithelial repair and infiltration of inflammatory cells. The host responds with the production of antibodies as well as intraepitheleal T lymphocytes. The infection may cause malabsorptive and secretory diarrhea. Management of infected patients includes maintenance of fluid and electrolyte balance. Patients with unresolved infection may be treated with macrolides and antimotility agents. A previous *Cryptosporidium*

infection does not confer resistance to reinfection although reinfection will result in fewer episodes of diarrhea. A CDC workshop panel focusing on the application of epidemiologic information on *Cryptosporidium* recommended making surveillance information available to the appropriate federal agencies, HMOs, hospitals, and others who play roles in maintaining the public health. Panel recommendations also included performing cohort analyses of outbreaks using information such as blood tests in populations where exposure to *Cryptosporidium* is likely. In addition, workshop participants suggested strategies to improve public health that included identifying methods for informing agencies, advocacy groups, and the public about risks for waterborne *Cryptosporidium* transmission and providing the public with information on dealing with a known or suspected contamination of a drinking water source.

VI. Risk Assessment

The International Life Sciences Institute (ILSI) Risk Science Institute (RSI) Pathogen Assessment Working Group (1996) defined pathogen risk assessment as a process that evaluates the likelihood of adverse human health effects following exposure to pathogenic microorganisms in a medium such as water. Until recently, most formal risk assessments on pathogenic microorganisms such as *Giardia* and *Cryptosporidium* have utilized a conceptual framework that was developed to assess risks due to chemical exposures; however, it is notable that 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 conditions that lead to propagation/multiplication 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 at acceptable public health risks (Rose *et al.*, 1997).

A. Experimental Human Data

The 1994 *Cryptosporidium* Criteria Document referred to human volunteer studies by DuPont and colleagues that were in progress at the time of manuscript preparation. The aim of this study was to determine the infectivity of *C. parvum* in healthy adults, in order to predict the likelihood of enteric infection following exposure to contaminated drinking water. The results of this study have since been published (DuPont *et al.*, 1995) and are presented in Table 4 of Section V-A. Among 29 subjects who were inoculated with 30 or more oocysts, 62% (18 subjects) became infected. Of those inoculated with 30 oocysts, 20% became infected, whereas of those inoculated with >1000 oocysts, 100% became infected. Illness lasted 58 to 87 hours, with 4 to 11 loose stools produced per day, suggesting 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} of 132 oocysts. The research team 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.

Follow-up analysis of the 18 individuals who presented with cryptosporidiosis infections indicated appreciable variation in the numbers of oocysts excreted and in the duration of excretion (Chappell *et al.*, 1996). Only 1 of the 7 volunteers who exhibited diarrhea had oocysts in every stool during the shedding period, and oocyst numbers in consecutive stool samples collected from individual volunteers varied by as much as 30 fold.

Hence, production of oocysts during infection may be intermittent, which may help to explain the observation that fewer than

50% of the individuals acquiring illness during waterborne outbreaks produce *Cryptosporidium*-positive stool samples when only one stool is examined.

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, versus 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).

B. Experimental Animal Data

A number of dose-response studies using monkeys, gnotobiotic lambs and several strains of mice were 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 the neonatal mouse was 60, which is approximately half of the ID_{50} required to produce infection in humans (132 oocysts). The relative similarity among infectious doses in mice and humans suggests that the mouse model is potentially useful in defining risks associated with human cryptosporidiosis.

C. Environmental Factors

1. Prevalence in Surface Waters

Cryptosporidium oocysts are more likely to occur in surface waters than in groundwater, as described in Section III of this document and in the 1994 *Cryptosporidium* Criteria document. Since the majority of source waters used for production of drinking water are surface supplies, and because these waters are more vulnerable to direct contamination from sewage discharges and runoff, the presumption has been that *Cryptosporidium* will likely be more common in these supplies. Wallis *et al.* (1996) found *Cryptosporidium* oocysts in 6.1% of raw sewage samples, 4.5% of raw water samples, and 3.5% of treated water samples in Canada. Analyses of raw sewage samples indicated that *Cryptosporidium* was present in more than 50% of samples where one or more liters was examined (Bukhari *et al*, 1997; Zuckerman *et al.*, 1997). Ong *et al.* (1996 a and b) studied the source of parasite contamination in different watersheds to assess the potential impact upon drinking water sources and found that water from rivers flowing through cattle pastures in British Columbia exhibited higher *Cryptosporidium* counts than did water from a protected watershed. Lisle and Rose (1995) reviewed 25 monitoring studies and found reports of *Cryptosporidium* in as much as 87.1% of the source waters (i.e., surface, spring, and groundwater samples not impacted by domestic and/or agricultural waste), with levels of oocysts as high as 4.7 per liter. LeChevallier *et al.* (1995) observed oocysts in 60.2% of surface waters tested in North America. Crockett and Haas (1995) investigated three water treatment plants located in a major metropolitan area where watershed monitoring was conducted over one year. They found that creeks which empty into a river were a primary source of parasites in the urban river-derived water. Each creek's parasite density was greatly influenced by suburban wastewater discharges, although the authors did not rule out other sources which might have influenced the microbial density in the river. In determining the implications of these

studies, however, it is important to note that erratic and insensitive oocyst detection methods may contribute to an underestimation of oocyst contamination levels in surface waters.

2. Oocyst Survival

The resistance of oocysts to inactivation is an important factor in determining the extent to which humans or reservoir/host animals can become infected; however, most detection methods for *Cryptosporidium* cannot distinguish between viable and nonviable oocysts. Furthermore, some detection methods may render oocysts nonviable due to chemical antagonism during sample processing. Additionally, the following practices and issues must be characterized more completely in order to more accurately evaluate the risk of contracting cryptosporidiosis: sewage discharges, watershed protection, agricultural practices, wildlife management, strain specificity (animal or human), and oocyst survival under various environmental conditions. The survival of oocysts under various environmental pressures has been evaluated by several groups and is described in Section III-G "Environmental Factors." The majority of these survival studies have relied upon animal infectivity or *in vitro* excystation to assess changes in oocyst viability in natural waters. A complete discussion on methods for the assessment of oocyst viability is also provided in Section VII-A- "Detection of *Cryptosporidium* in Water."

3. *Cryptosporidium* in Drinking Water

Identification of the specific pathogen and route of infection is an early step in the risk assessment process. The primary route of human infection by *C. parvum* involves ingestion of contaminated drinking water and food (Casemore, 1990); other routes of transmission are described in Section II-D. One of the difficulties in conducting a risk assessment of *Cryptosporidium* lies in the uncertainty associated with the level of infectious

oocysts in drinking water supplies. There are also viability, infectivity, and specific epithet issues. Surveys such as those described in Section III-B indicate the numbers of oocysts that may occur in drinking water; however, the impact of these oocyst levels may be underestimated, given the number of gastrointestinal illnesses that occur each year for which the etiology is undetermined.

Nahrstedt and Gimbel (1996) examined the influence of various factors contributing to the uncertainty associated with the estimation of *Cryptosporidium* and *Giardia* concentrations in water samples. A statistical model was designed using experimental data. The model provides reliable estimates of the oocyst/cyst concentration in a given water body from which a representative sample has been taken and analyzed. Their discussion of the effects of errors in detection methods may lead to improved analytical methods and a better understanding of results obtained from current detection methods.

D. Epidemiologic Considerations

The USEPA estimated in 1993 that approximately 155 million people may be exposed to *Cryptosporidium* in contaminated water every year; however, the estimated population at risk cannot be reconciled with the reported numbers of infected individuals, even when correction factors for asymptomatic infections and underestimated environmental levels are included (USEPA, 1994). Factors contributing to the disparity between the environmental occurrence data and the clinical data are outlined in the 1994 document; additional factors are described in Section V-B of this document. It remains quite problematic to generate accurate estimates of the risk of acquiring cryptosporidiosis.

Despite the limitations, the incidence of cryptosporidiosis in the United States is typically assessed through surveillance reports and the documentation of outbreaks in the published literature. The CDC currently maintains a surveillance system for cryptosporidiosis that is aimed at collecting information on both outbreak- and non-outbreak-related cases. Cases are reported using standard forms which originate from state and local health departments, but the agency also receives updates from federal agencies and occasionally from private physicians. While cryptosporidiosis is not a reportable disease in all states (CDC, 1994), it was designated as notifiable at the national level as of January 1, 1997. 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 determining the true incidence of cryptosporidiosis in the U.S.

A number of reports describe the severe effects of cryptosporidiosis in children, particularly in malnourished infants (Molbak *et al.*, 1994; Griffiths, 1998). However, it is generally difficult to determine if malnourished children are at higher risk of chronic cryptosporidiosis due to immune suppression, or if cryptosporidiosis is an independent risk factor for becoming malnourished (Griffiths, 1998). Reports from the U.K. show the occurrence of cases to be highest among children less than five years old (Atherton *et al.*, 1995). Other groups at risk for cryptosporidiosis are secondary contacts, farm workers (Lengerich *et al.*, 1993), immune-suppressed individuals, those living in institutional settings such as group homes and orphanages (Heald and Bartlett, 1994), and international travelers who visit regions where cryptosporidiosis is endemic. There is little evidence that risks differ between genders (Meinhardt *et al.*, 1996).

E. Risk Assessment Models

Since *Cryptosporidium* monitoring does not presently provide a true picture of the number of infectious particles and the efficacy of oocyst removal from treated drinking water, risk calculations involve many uncertainties. In order to develop risk estimates for specific pathogens such as *Cryptosporidium*, reliable dose-response data are required. The human dose-response data currently available are limited to the studies of DuPont *et al*. (1995), Chappell *et al*. (1996), and Okhuysen *et al*., (1999); however, an exponential dose-response model has been developed, based upon the data set and a number of assumptions governing the Milwaukee epidemic of cryptosporidiosis (Haas, 1994). This model describes the probability of infection given exposure (P_I) as follows:

Equation 1. $$P_I = 1\text{-}e^{-rN}$$

The values of **r** and **N** represent the fraction of ingested oocysts which must survive to establish infection and the daily exposure, respectively. A value of **r** specific for *Cryptosporidium* has been derived (r=0.0047). Oocyst concentrations were derived for exposures ranging from 1 to 30 days and P_I ranged from 0.14 to 0.52. Exposure values predicted according to this model ranged from a minimum of 0.16 oocysts per liter (P_I=0.14) to a maximum value of 79 (P_I=0.52).

Cryptosporidium concentrations in the Milwaukee water supply were estimated by considering the numbers of oocysts present in ice produced during the epidemic and correcting for losses associated with poor analytic recovery efficiencies. According to the exponential model, *Cryptosporidium* exposure during the epidemic ranged from 0.6 to 1.3 oocysts per liter. Haas (1994) also applied the risk assessment model to consider data

from previous water monitoring studies and calculated that the annual risk of contracting cryptosporidiosis in the United States may range from 1 in 100,000 to 4 in 1,000.

Perz *et al.* (1998) applied a risk assessment approach to examine the role of tap water in waterborne cryptosporidiosis. This model was based upon the assumption that clinical infection results from exposure to a single oocyst, and it utilized a theoretical *C. parvum* density in drinking water of 1 oocyst per 1,000 liters. Uncertainties in the model were analyzed by considering ranges and distributions among the input variables. The number of annual *Cryptosporidium* infections (I_j) was estimated according to the following relationship:

$$I_j = C \bullet POP_j \bullet Q_j \bullet r_j$$

where C = concentration of *C. parvum* per liter of water
j = population subgroup (categorized by age and AIDS status)
POP_j = number of persons in the exposed subgroup
Q = annual water intake (liters per year)
r = 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 the 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). Therefore, improvements in *Cryptosporidium* monitoring techniques for drinking water will improve future risk assessment efforts.

The ILSI workgroup (1996) has developed guidance for an infectivity model that may also be useful when sufficiently sensitive detection methods become available. Since exposure analysis for humans and animals is

an essential step in risk assessment, developing animal systems that can be used to estimate infectivity will be essential for modeling, because detection of oocysts without knowledge about their infectivity is inadequate. Varga *et al.* (1995) reported on a model using *C. baileyi* infection in chickens. The authors developed a quantitative method to assess oocyst shedding which was based on the rather slow sedimentation of oocysts. A threshold of sensitivity of between 5,000 and 10,000 oocysts per gram of feces was reported for the technique. Triplicate assays over a wide range of oocyst concentrations (i.e., 2,500 to 1.25 x 10^6) were in good agreement. Considering the level of oocysts excreted by infected humans, this model system appears to have adequate sensitivity for demonstrating infection. This model's sensitivity threshold may be adequate for assessing stool samples of infected individuals, but it is inadequate for monitoring oocysts in water samples for potential infectivity; thus, further development of sensitive infectivity models is needed.

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). In order to assess exposure, the progression of the pathogen from the river water to the tap water was broken down into the following stages: oocyst counts in source water (corrected for detection method), source water concentration, removal by storage, and removal by treatment. Each stage was analyzed successively by means of statistical models. The daily ingested dose, which was calculated by means of a Monte Carlo simulation, was a single distribution for the population as a whole because data for various subpopulations were not available. A Beta Poisson model was developed for the dose-response assessment using experimental data from infection of human volunteers with *C. parvum.* 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).

F. Federal Regulations

Since the 1994 *Cryptosporidium* Criteria Document was published, *Cryptosporidium* is now specifically regulated by the federal government as a primary drinking water contaminant. The federal regulatory activity associated with *Cryptosporidium* in drinking water and its threat of waterborne disease was prompted primarily by the 1996 Amendments to the Safe Drinking Water Act. The most significant promulgated and proposed rules addressing *Cryptosporidium* since 1994 are the Information Collection Rule, the Interim Enhanced Surface Water Treatment Rule, and the Long Term I Enhanced Surface Water Treatment and Filter Backwash Rule.

On May 14, 1996 the USEPA promulgated the Information Collection Rule (USEPA, 1996a). The rule required those water utilities serving more than 10,000 people to test source water and finished water for an 18-month

period (from July, 1997, to December, 1998). The monthly testing included a variety of analytes such as coliforms, turbidity, and *Cryptosporidium.* The rule was primarily a research effort and the USEPA is using the information collected during the testing period for the development of future rules. The data generated from the Information Collection Rule is now available to the public through Envirofacts (http://www.epa.gov/enviro/html/icr/icr_query.html).

The Interim Enhanced Surface Water Treatment Rule was promulgated on December 16, 1998 (USEPA, 1998). The rule applies to water utilities using surface water, or groundwater under the direct influence of surface water, and serving more than 10,000 people. The rule 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. In addition, the Interim Enhanced Surface Water Treatment Rule includes *Cryptosporidium* in the watershed control requirement for unfiltered public water systems. This rule was designed to establish physical removal efficiencies and to minimize *Cryptosporidium* levels in finished water. 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 in late Spring 2001. The Long Term I Enhanced Surface Water Treatment 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 Enhanced 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.

G. Summary

Environmental risk assessments have historically relied upon a conceptual framework based upon exposure to chemical pollutants and are generally considered inadequate for pathogen risk assessment. Although most human populations are assumed to be at risk for cryptosporidiosis at least to some degree, it has been difficult to collect accurate figures describing the prevalence of infection in humans due to limitations in public health reporting systems and due to incomplete characterizations 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. Data from animal infectivity studies suggest that infectious doses may be lower (e.g., ID_{50} of ~60 oocysts). Risk models have been developed to assess the probability of cryptosporidiosis infection based upon assumptions governing 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 1,000,000.

VII. Analysis and Treatment

A. Analysis of Water

The Information Collection Rule-modified American Society of Testing and Materials method (ASTM ICR) was described in the 1994 *Cryptosporidium* Criteria Document. The method, which detects both *Cryptosporidium* and *Giardia*, is tedious and requires high levels of technical expertise. While reproducible and sensitive for *Giardia* detection, the method is not reproducible between laboratories and suffers from low sensitivity for *Cryptosporidium* detection (Clancy *et al.*, 1994; USEPA, 1996b). The current standard method for monitoring *Cryptosporidium* in water is EPA's Method 1622 (USEPA, 1999b).

The accuracy and reproducibility of method development and comparison studies were questioned by Klonicki *et al.* (1997). The authors noted that vital information regarding oocyst source, purification method, age, storage conditions, and enumeration method was inadequately cited in most publications. Further studies compared three enumeration methods, hemacytometer, membrane, and well slide, and showed statistically significant differences among the methods. The authors demonstrated the effect of counting method variability on recovery values and stressed the importance of standardizing method comparisons and providing adequate information in publications to allow valid comparison studies.

LeChevallier *et al.* (1995) investigated numerous methodological variations in the collection, elution, and concentration portions of the assay to determine their influence on recovery of *Cryptosporidium* in seeded tap water and Mississippi River water samples. The authors tested multiple filter types, centrifugation speeds,

density gradient modifications, elution buffer pH values, and centrifuge bottle types. Although they identified several sources of oocyst loss, their modifications resulted in variable recovery (7-129%).

Before detection data can be applied in a meaningful way, it must be adjusted to reflect the limits of the methodology used to collect it. For example, if the method can detect 1,000 oocysts in 100 L of water and a sample contains only 50 oocysts, a result showing 0 oocysts could be obtained. The results, therefore, must be reported as "less than 1,000 oocysts." Harris (1995) developed a method detection limit for indirect immunofluorescence assay (IFA) analysis of *Cryptosporidium* and *Giardia*. The $MDL_{0.95}$ in this study is defined as the minimum concentration that the procedure can analyze with 95% confidence that the organism will be detected.

Each water type presents unique problems in collection, concentration, isolation, and staining techniques. Organic and inorganic particulates present in the water can clog filters or interfere with other portions of the analysis such as clarification or antibody staining. Additionally, the ASTM ICR immunofluorescent staining methods do not give information regarding viability, infectivity, and speciation which is essential to assessing the threat to public health. The reader is advised to review Table 3.19 in Frey *et al*. (1998) for a complete synthesis of the research status of *Cryptosporidium* detection methods. Current efforts to develop improved *Cryptosporidium* collection, concentration, and detection methods are described below.

l. Collection of *Cryptosporidium* from Water

Filtration-based concentration methods

The 1994 *Cryptosporidium* Criteria Document described a filtration method using polypropylene wound yarn filter with a 1- m porosity. This collection method can be used for large volume samples with varying turbidity. LeChevallier *et al.* (1995) tested 10 cartridge filters varying in composition (polypropylene, nylon, rayon, and cotton) and porosity (0.5 and 1.0 m) for removal of *Cryptosporidium*- and *Giardia*-sized particles. Although retention of 3- and 7- m particles was greater using filters with a 0.5 m porosity, they tended to clog, limiting the amount of water that could be filtered. The use of cotton, nylon, and rayon filters led to the most efficient removal of *Cryptosporidium* and *Giardia*-sized particles. The authors tested the filters using 1 gallon (3.78 L) volumes of water. Wound fiber filters may not necessarily be superior to wound filters for samples greater than 1 gallon in volume. To further minimize losses during filtration, the filter housing was matched with the filter, and a screw press was used to wring the filters. Concentration of the eluate was best performed at centrifuge speeds of 6,700 to 10,000 x g.

Also described in the 1994 *Cryptosporidium* Criteria Document was the use of cellulose acetate membrane (CAM) filters. Nieminski *et al.* (1995) compared recovery rates of a method using CAM filters to the ASTM ICR method using wound yarn filters. Prior to filtration by either method, *Cryptosporidium* and *Giardia* were spiked into environmental water samples varying in quality and turbidity. Cyst and oocyst recoveries decreased with increasing water turbidity, regardless of the filter type. Overall, the cellulose acetate method gave higher recoveries; however, because the parasites were stained on polycarbonate filters, microscopic confirmation was not possible. Therefore, the authors recommended the use of the ASTM ICR method for environmental sample

analysis and the cellulose acetate method for spiking studies. Adlom and Chagla (1995) modified the CAM method by including an acid dissolution step following filtration. This modification resulted in a 70.5% recovery of oocysts spiked into 3 liters of treated municipal water. Graczyk *et al.* (1997b) used cellulose acetate filters, followed by filter dissolution and ASTM ICR method processing to test recovery of *Cryptosporidium* from spiked drinking water. The overall mean recovery rate was reported as 77.7%. Further studies by Graczyk *et al.* (1997b) indicate that the acetone dissolution step does not compromise viability or infectivity.

EPA's Method 1622 requires a capsule filter (USEPA, 1999b) (e.g., Envirocheck™ capsules); these filters contain a pleated polysulfone membrane with a 1- m porosity. Envirocheck™ is a 6-cm-diameter by 21-cm-long capsule with a surface area of 1,300 cm^2. Clancy (1997) compared throughput and recovery rates of this capsule filter with those of polycarbonate membrane filters, vortex flow filtration, and cellulose acetate membrane filters which were dissolved post-filtration. All four filters were challenged with 10 liters of municipal raw and finished waters. The cellulose acetate membranes and polycarbonate membranes were blocked at 8 and 2.5 liters, respectively, at a raw nephlometric turbidity unit (NTU) of 5. The polymer vortex flow and Envirocheck™ capsule filters were able to process the entire 10 liters of raw water and gave recovery rates of 11-57% and 8-78%, respectively. In finished waters from five utilities, the vortex flow recovered 18-69% of the seeded oocysts, while the capsule filter recovered 45-117%. The researchers concluded that the capsule filter performed best with the various water matrix conditions tested. Other membrane filters composed of glass fiber have been evaluated but shown to be of poor integrity (Whitmore and Carrington, 1993).

Centrifugation-based concentration methods

Vortex flow filtration (VFF) is a centrifugation-based filtration method in which a water sample is passed through a cylindrical membrane filter rotating at high speed within a second cylinder. The rotation sets up forces that scrub the membrane surface and prevent blockage by particulates. The liquid phase (permeate) crosses the filter while the particulate-containing phase (retentate) is continuously recirculated and concentrated. Whitmore and Carrington (1993) evaluated the ability of a VFF apparatus with a 0.45- m polysulfone membrane cartridge filter, followed by a clarification step using density gradient centrifugation, to concentrate *Cryptosporidium* from spiked bore hole or river water samples. The VFF device recovered between 30 and 40% of the seeded oocysts. Fricker (1997) reported greater than 60% recovery using VFF in seeded river water samples.

Whitmore and Carrington (1993) evaluated cross-flow filtration for recovery of oocysts in clean water. This apparatus pumps water across a set of alumina filters in a parallel series. In this study, the retentate, typically 150-200 ml, was collected, centrifuged, resuspended in phosphate-buffered saline (PBS), and counted using a hemacytometer under Nomarski differential interference contrast (DIC) illumination. The authors noted that this method is rapid and recovers 70% of the seeded oocysts in small-volume samples. Studies using larger volume samples recovered 40%. The authors suggested that more effective cleaning or replacement of the filters between runs may result in higher recovery rates. The device produces small volumes of retentate, which facilitate further concentration, is compact, and can be sterilized.

Researchers at Marshfield Clinic in Marshfield, Wisconsin have developed a continuous centrifugation method to concentrate parasites from water (Borchardt and Spencer, 1996). This method uses a blood cell separator,

operating on the principle of channel-type centrifugation, to concentrate oocysts from water samples. Recoveries over a range of water turbidities, oocyst concentrations, and water volumes were reported to be 78-101% .

Concentration using flocculation

Calcium carbonate flocculation has also been used to concentrate *Cryptosporidium* oocysts in up to 10 L of water. The method, developed by Vesey *et al.* (1993b), uses calcium chloride and sodium bicarbonate in a high pH solution to crystallize organic particles. The crystals are allowed to settle, the supematant is discarded, and the calcium carbonate precipitate is dissolved with sulfamic acid. Vesey *et al.* (1993b) reported recoveries greater than 68% with this method. Subsequent analyses of this method by Shepherd and Wyn-Jones (1995 and 1996) were in agreement and reported that calcium carbonate flocculation consistently gave higher recoveries than cellulose acetate membrane and cartridge filters. However, calcium carbonate flocculation is not recommended for drinking water analysis when viability is important. Studies by Robertson *et al.* (1994) showed significant viability reduction, as determined by vital dye staining (see Viability, VII-A-2) following a 4-hour exposure to a pH of 10.

Clarification by density gradient centrifugation

Density gradient flotation methods are commonly used to clarify samples and concentrate oocysts prior to detection. Centrifuge speed and time and the density of the solution vary among laboratories using this method. LeChevallier *et al.* (1995) reported that a Percoll sucrose gradient with a specific gravity of 1.15 was 67% better than a gradient with a specific gravity of 1.0 for recovery and concentration of oocysts. A gradient with a

specific gravity of 1.3 did not clarify the sample and interfered with microscopic analyses. This study also indicated that flotation selects for empty oocysts while live, intact oocysts sink to the bottom of the gradient rather than floating to the upper fraction (LeChevallier *et al.*, 1995). Shepherd and Wyn-Jones (1995) reported significant decreases in recovery (>50%) when sucrose flotation techniques were included in detection methods.

Flow cytometry

A flow cytometer is a laser-based instrument that analyzes particles in a liquid suspension on a particle-by-particle basis. It can differentiate and physically separate (sort) particles based on their size, internal complexity, and fluorescence. Flow cytometry with cell sorting (FACS) is used routinely in the U.K. and Australia for detection of *Cryptosporidium* and *Giardia* in environmental samples (Vesey 1993a,1994). Briefly, a concentrated portion of the sample is incubated with a fluorochrome-conjugated monoclonal antibody that binds a portion of the oocyst wall, causing the organism to fluoresce. The instrument analyzes the particles and electrically charges those selected by the operator based on a signal (e.g., fluorescence). The charged particles are pulled out of the sample stream using oppositely charged electrical plates and deflected onto a microscope slide. The slides, free of the debris which can obscure oocysts on a membrane filter slide, can be read in 5 to 10 minutes as opposed to the 90 to 120 minutes typically cited for membrane analysis. In studies published by Vesey *et al.* (1994), FACS detected greater than 92% of the *Cryptosporidium* and *Giardia* in seeded river and reservoir samples. Additional work incorporating a calcium carbonate flocculation concentration step prior to FACS reported 64.1% and 62.7% recovery of the oocysts seeded in filtered and raw waters, respectively (Veal, 1997). Studies by Hoffman *et al.* (1997) with a variety of environmental samples reported that FACS detected almost three times more *Cryptosporidium*-positive samples than membrane immunofluorescence assay (IFA) (94.1% vs. 35.3%, respectively) and an equal number of *Giardia*-positive samples. This technique has been

repeatedly shown to be superior to traditional membrane IFA analysis (Danielson, 1995; Compagnon, 1997). Researchers at Macquarie University in Australia, working with a flow cytometer manufacturer, have modified the traditional flow cytometer to optimize it for water quality analysis. This type of flow cytometer is not currently available in the U.S. The FACS method provides increased sensitivity and requires less time, expense, and experience than the ASTM IFA method. Additionally, this method can, with no extra effort, simultaneously detect *Giardia*. Disadvantages of this method include the initial expense of the instrument ($150,000-200,000) and the level of flow cytometry expertise required with the non-optimized models.

Immunomagnetic separation

Method 1622 uses immunomagnetic separation (IMS) to separate oocysts following a filtration step (USEPA, 1999b). IMS concentrates *Cryptosporidium* oocysts by using magnetic beads coated with an anti-*Cryptosporidium* antibody. Following elution, the sample is incubated with magnetic beads that bind the oocysts. The solution is inserted into a magnetic particle concentrator that binds the magnetic bead-*Cryptosporidium* complex. After the supernatant is decanted, the beads are released from the magnet. Oocysts are dissociated from the magnetic particles using an acid wash, neutralized with base, and subjected to analysis. This method was evaluated for *Cryptosporidium* by Robertson and Smith (1992). Later efforts to develop a *Giardia* detection system were pursued by Bifulco and Schaeffer (1993) who reported an 82% recovery rate for *Giardia* in waters of varying turbidities. Campbell *et al.* (1997) spiked laboratory-grade and turbid water samples with *Cryptosporidium* and concentrated them using either the ASTM IFA, the U.K. Standing Committee of Analysts method (SCA), or IMS. IMS gave the highest recoveries from either water type. Clean water recoveries using IMS were 97.4%, while the ASTM IFA and SCA methods recovered 26.9% and 19.3%, respectively. Turbid water recoveries were 65.8% using IMS, 5.4% with the ASTM IFA, and 11.7% with SCA.

A five-site evaluation comparing IMS to FACS and a modified SCA reported the IMS method consistently resulted in higher oocyst recoveries than FACS and SCA methods in low turbidity waters (Campbell and Smith, 1997). However, efficiency of the IMS method was decreased in high turbidity water samples. Flow cytometry showed the greatest recoveries in higher turbidity waters. When Fricker *et al.* (1997) evaluated the IMS procedure in water samples seeded with 100 oocysts, recovery rates ranged from 71-120%.

Panning

Panning is a technique originally developed to isolate specific cell types from a mixed cell population. An antibody raised to the target is attached to a solid substrate and incubated with the target cell-containing suspension. During the incubation period, the target cell or organism is bound by the antibody and the remaining cells or debris can be washed off. Direct and indirect panning methods were tested by Stone (1997) to concentrate *Cryptosporidium* oocysts during the clarification stage. The authors recovered 50% of the oocysts seeded into Hank's balanced salt solution by direct panning and 20% of the seeded oocysts using indirect panning.

2. Detection of *Cryptosporidium* in Water

IFA

Direct and indirect immunofluorescent antibody detection methods facilitate the visualization of oocysts which may be obscured by debris in environmental samples. The indirect immunofluorescence assay (IFA) (Fout *et al.*, 1996), described in the 1994 *Cryptosporidium* Criteria document, remains the most widely used detection method. The test, however, does not provide information regarding viability, infectivity, or species. The HydroFluor Combo staining reagents have been shown to cross-react with various algal species (Rodgers *et al.*, 1996), and an experienced microscopist is essential for accurate and reliable examination.

Well slide staining

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, 1999b). Well slide staining has been evaluated as an alternative to membrane IFA staining (Frederickson *et al.*, 1995). In a five-site side-by-side comparison, *Cryptosporidium* recoveries using the well slide method were in excess of 50% higher than those obtained using traditional membrane staining. Additionally, the well staining procedure took 50% less time to perform. The authors suggested the physical forces of the vacuum used in membrane IFA staining may result in destruction of cysts and oocysts. Microscopic examination of the contents of the apparatus used for membrane IFA staining revealed a 78% loss of *Cryptosporidium*, with 2% remaining intact. *Cryptosporidium* oocysts have been shown to be compressible (Li *et al.*, 1995) and may slip through filters with pore sizes smaller than their 4-6 m diameter.

Enzyme immunoassays

Traditional enzyme immunoassays (EIA) can provide rapid detection of oocysts with little tedium. While these assays have been used clinically, their use in environmental analyses is not common. Several papers have shown that EIA can be used to analyze environmental samples (Siddons, 1991; Chapman, 1990; Gracyzk *et al.*, 1996b). In one instance, Siddons (1991) reported a positive EIA detection of one oocyst. Chapman and Rush (1990) reported EIA sensitivity equal to microscopic examination in both environmental and human samples. De la Cruz and Sivagansen (1994) tested two *C. parvum* EIA kits for detection of oocysts in buffered saline and river water. The authors found the kits were capable of detecting <10 oocysts; however, results were variable with fixed and unfixed organisms. Both EIA kits cross-reacted with algae. Gracyzk *et al.* (1996b) compared the specificity of the Prospect T™ enzyme-linked immunosorbent assay (ELISA) to that of the HydroFluor Combo antibody assay used in the ASTM ICR method and the Merifluor direct stool kit antibody. Their results showed the ELISA gave a positive reaction with only 6 of 25 non-*C. parvum* isolates tested, while the HydroFluor Combo and Merifluor direct antibodies each cross-reacted with 19 of such isolates.

Molecular methods: polymerase chain reaction assays

Several reports describe various applications of the polymerase chain reaction (PCR) for the detection of *Cryptosporidium* in drinking water (Rochelle *et al.*, 1997a and b; Johnson *et al.*, 1995; Wagner-Wiening and Kimmig, 1995; Filkorn *et al.*, 1994; Johnson *et al.*, 1993). These methods rely upon *in vitro* enzyme-mediated amplification of *Cryptosporidium*-specific nucleic acids in order to facilitate identification in water samples. In theory, this technique should offer unmatched endpoint sensitivity as well as the possibility of distinguishing subtle differences among discrete strains of parasites. A number of techniques aimed at distinguishing viable

and nonviable oocyst populations are also reported. These applications are summarized below. It is important to emphasize that, due to the enzymatic nature of the PCR process, these assays may fail due to inhibition of enzyme activity caused by compounds commonly found in natural waters. Additionally, the majority of the documented studies have been limited to evaluations of seeded water samples rather than actual comparative field trials. Hence, the application of these techniques toward the detection of *Cryptosporidium* in environmental water samples should be considered developmental.

Johnson *et al.* (1995) reported a PCR protocol to detect *Cryptosporidium* in environmental samples, based upon oligonucleotide primers specific to a portion of the small 18S ribosomal RNA. Detection sensitivities of 1 to 10 oocysts were achieved in purified oocyst preparations; however, the detection sensitivity in seeded environmental samples was up to 1,000-fold lower. Poor endpoint sensitivity was at least partially offset by a concentration step using either flow cytometry or immunomagnetic capture and by oligoprobe hybridization using a chemiluminescent technique. These methods were applied to confirm the presence of oocysts in water samples from the Milwaukee outbreak of cryptosporidiosis, and the results of the PCR assays were comparable to those observed when immunofluorescence methods were used.

Rochelle (1997b) evaluated four pairs of previously published primers aimed at the specific detection of *C. parvum.* Detection sensitivities ranged from 5 to 50 oocysts in seeded environmental samples when PCR was followed by oligoprobe hybridization, with some primer pairs offering species specificity while others were only genus-specific. Successful multiplex reactions aimed at the simultaneous detection of *G. lamblia* and *C. parvum* were evaluated and demonstrated the utility of PCR for the detection of waterborne parasites. However,

no primer combinations were identified which exhibited the ideal combination of sensitivity, specificity, and compatibility with multiplex reactions, and several primer sequences previously reported failed to amplify their targets.

Since PCR is capable of detecting the genetic material of both live and dead microorganisms, a number of studies have targeted unique sequences to distinguish viable from nonviable oocysts. Wagner-Wiening and Kimmig (1995) applied PCR to detect *Cryptosporidium* by targeting a large DNA fragment specific to *C. parvum.* In order to differentiate between live and dead oocysts, this group reported the practice of applying an excystation protocol prior to PCR and targeting sporozoite DNA to ensure that amplified material was associated only with viable oocysts. Endpoint sensitivity as low as 100 sporozoites was observed and was reduced to 10 sporozoites when nested PCR was practiced. Filkhorn *et al.* (1994) also evaluated RNA-based measurement of viable oocysts by practicing excystation prior to PCR. To preclude spurious contamination caused by the presence of DNA in nonviable parasites, a DNase enzyme was applied to digest free DNA, leaving only free RNA from viable oocysts.

Stinnear *et al.* (1996) described a reverse transcription-PCR (RT-PCR) detection method specific for *C. parvum* which can detect single viable oocysts and is based upon the assumption that only viable oocysts are metabolically active and will produce messenger RNA (mRNA). Since mRNA exhibits an extremely short half-life in living cells (and perhaps even shorter outside of the protective environment of the oocyst wall), only the mRNA from viable oocysts will be captured and amplified.

PCR has also been used to track *Cryptosporidium* reductions during water treatment. Mayer and Palmer (1996) evaluated several methods to identify *Cryptosporidium* oocysts in sewage and to determine reductions during wastewater treatment. The nested PCR technique described above was compared to a modified ASTM method to track reductions during treatment, and strong correlations were observed, with approximately 2 $\log_{10}$ *Cryptosporidium* reductions observed. The authors concluded that the PCR method was preferable due to a substantial reduction in sample collection and processing during analysis.

Molecular methods: cell culture-PCR

At least one integrated approach has been reported that utilizes tissue culture, PCR, and *in situ* PCR (IS-PCR) to assess seeded natural water concentrates for the presence of infectious oocysts (Rochelle, 1997a). This technique offers the possibility of screening out noninfectious oocysts, since only the infectious oocysts will develop during the initial phase of amplification in human adenocarcinoma cells. Preliminary experiments suggest that IS-PCR may offer quantitative detection of infectious oocysts in natural water concentrates.

Molecular methods: strand displacement amplification

A strand displacement assay for the detection of *Cryptosporidium* in natural waters samples has been reported to overcome the comparatively long cycle times associated with PCR (Blassak *et al.*, 1996). This method is based upon the selective replication of a single DNA strand while the other parental strand is displaced from the template, with colorimetric detection of oocyst DNA under a microplate format. A horseradish peroxidase-streptavidin conjugate is used for color development. The results of the strand displacement assay correlate well with evaluation of acid-fast slides of fecal samples.

Laser scanning microscopy

The ChemScan RDI is a laser scanning device linked to an epifluorescent microscope with a motorized stage. Sample analysis includes a filter staining procedure, followed by automated scanning with the ChemScan instrument. The instrument digitizes the location of fluorescent objects, allowing for quick confirmation. This instrument has the advantage of being highly automated and more sensitive than FACS and IMS methods in samples with colloidal clay (Reynolds *et al.*, 1997). The recovery rate, minimal detection limit, and oocyst spike concentration were not specified. Disadvantages of this method include the cost of the instrument ($200,000-300,000), use of membrane filters which have been shown to contribute to oocyst loss (Frederickson *et al.*, 1995), and the inability to perform any light microscopy techniques for visualization of internal cytoplasm or sporozoites.

Miscellaneous methods

Campbell *et al.* (1993a) contrasted flow cytometry and a slow scan cooled-charge couple device (CCD) for detection of *Cryptosporidium.* The authors discussed the advantages of the CCD, including its ability to simultaneously assess viability by DAPI staining; however, a comparison of results was not provided. The need for sophisticated and currently unavailable software was noted by the authors.

Campbell *et al.* (1993b) also evaluated enhanced chemiluminescence for detection of *Cryptosporidium* in 21 environmental samples previously assayed by microscopy. The oocysts were labeled with a fluorescein isothiocyanate (FITC)-conjugated anti-*Cryptosporidium* antibody, followed by a biotin-conjugated anti-FITC antibody and streptavidin-peroxidase. Statistical analysis revealed no difference in results obtained with the

enhanced chemiluminescence assay and microscopy. The authors reported that the chemiluminescence method is faster than the currently used microscopic method; however, visual confirmation was still necessary.

The electrorotation assay (ERA) relies on the principle that small particles can be induced to rotate in the presence of a rotating electric field. The rate of rotation is due to the field and surface charge of the particles. Jakubowski *et al.* (1996) described a proprietary electrorotation assay in which oocysts are attached to an antibody-coated magnetic bead and placed in a filter electrode assembly. The assembly is placed under a microscope, connected to an electric field generator, and the number of rotating oocysts are enumerated. This method may differentiate between viable and non-viable oocysts, based on differences in their rotation rates. Oocyst recovery rates have been reported to range from 30-95%; however, the efficiency of this method is dependent on the type and characteristics of the water. A major disadvantage to ERA is that magnification can only be performed up to 400× due to the thickness of the ERA unit.

Method 1622

Recognizing the need for an improved *Cryptosporidium* detection method, the USEPA initiated an effort to identify new and innovative technologies for protozoan monitoring and analysis. Following a comprehensive evaluation of existing and emerging technologies, the USEPA Office of Water developed an initial draft of Method 1622 in December 1996 (EPA-821-R-97-023). This method has been validated and is now used as a standard procedure (EPA-821-R-99-001) for collection and quantification of *Cryptosporidium* oocysts from water samples.

With Method 1622, 10-L sample is shipped to the laboratory, where it is filtered through a polysulfone capsule filter. Following filtration, the capsule is filled with eluting solution and shaken on a wrist action shaker for approximately 5 minutes. The capsule is drained and the elution procedure is repeated. The combined eluate is concentrated by centrifugation, reconstituted to 10 ml, and subject to IMS as described previously in Section VII.A.1. The solution is stained with anti-*Cryptosporidium* antibodies using the well slide method described previously in this section.

Viability determinations

The public health significance of *Cryptosporidium* relates primarily to the ability of this parasite to initiate infection in humans and animals. Although the gold standard among infectivity assays remains the animal model, the high costs and ethical considerations associated with assays using animals preclude their routine use. Additionally, these methods do not offer adequate sensitivity for testing of environmental samples when the level of oocyst contamination may be on the order of 1 or 2 oocysts per L. Differences in pathogenesis among humans and animals have also called into question the applicability of animals in providing an accurate reflection of the number of *Cryptosporidium* required to cause cryptosporidiosis in humans. Nonetheless, innovative viability assessment methods for *Cryptosporidium* oocysts are inevitably compared to animal models, primarily using mice, and these methods are described below.

The *in vitro* excystation (IVE) method estimates infectivity by determining the number of potentially infectious oocysts based on simulating the conditions of the mammalian gastrointestinal tract. Oocysts exposed to an acid pretreatment and bile salts at elevated temperatures will release progeny sporozoites (excyst), whereas

metabolically inactive (nonviable) oocysts will fail to excyst under such conditions. Excystation is tracked by direct microscopic visualization of treated samples and their comparison to the initial untreated population. Black *et al.* (1996) and Belosevic *et al.* (1997) indicate that the IVE assay may significantly overestimate the true infectivity of oocysts treated with chemical antagonists, compared to the results of animal infectivity studies. Although the IVE assay is relatively time-consuming and is generally not applicable toward tracking reductions during water treatment (which may exceed several orders of magnitude), Vesey *et al.* (1997) have reported an adaptation of this method that uses flow cytometry to increase the sensitivity and throughput, and they have observed good agreement with the microscopic method.

The *Cryptosporidium* Criteria Document (1994) described the early work of Campbell *et al.* (1993b), which focused on vital dye staining. This group evaluated the application of dyes which are preferentially absorbed by viable oocysts, compared to the technique with IVE, and strong correlations were observed. This work was confirmed by Bukhari (1995) who compared vital dye staining and IVE of oocysts concentrated using several different methods. Subsequent studies by Black *et al.* (1996) indicated that vital dye staining correlated well with IVE but had a tendency to overestimate infectivity of ozone-treated oocysts when compared to animal infectivity. Jenkins *et al.* (1997) confirmed that vital dye staining tends to overestimate infectivity relative to animal infectivity, but they supported its use as an economical, user-friendly method that provides information on the effects of stresses on the surface of oocysts over time. Belosevic *et al.* (1997) evaluated 14 nucleic acid stains as indicators of *Cryptosporidium* viability and compared the staining to both animal infectivity and IVE assays. Both heat-treated and chemically inactivated oocysts were consistently stained with novel stains (SYTO-9, SYTO-59, and hexadium). This staining correlated well with animal infectivity but not the IVE assay. The authors also developed an IFA viability assay that relies only on propidium iodide (PI) for viability

assessment; however, extraneous factors such as aldehyde fixation may inhibit uptake of PI and falsely elevate the numbers of viable oocysts (Campbell *et al.*, 1993c).

Various infectivity assays have been described using tissue culture methods to assess the infectivity of *C. parvum* (Upton *et al.*, 1994a; Upton *et al.*, 1994b; Upton *et al.*, 1995; Rochelle *et al.*, 1997a; Slifko *et al.*, 1997). These methods track the development of progressive *Cryptosporidium* infections in cell cultures. Evaluation of infected cultures can be facilitated by conducting ELISAs following a 1-2 day incubation, and then scoring the extent of infection (and hence number of viable oocysts present in the original inoculum) by spectrophotometry in an automatic plate reader. A detection sensitivity of approximately 100 oocysts has been described (Upton *et al.*, 1994a and b). Slifko *et al.* (1997) describes a semi-quantitative method which relies upon staining infected tissue cultures with fluorescent antibody and then tracking the numbers of infectious foci using epifluorescence and DIC microscopy. An adaptation of the tissue culture method using PCR (Rochelle *et al.*, 1997a) is described above in Section VII-A-2 "Molecular methods: polymerase chain reaction assays."

3. Assessment of Laboratory Testing Capabilities

Detection of *Cryptosporidium* and *Giardia* and the ability to distinguish them from other organisms of comparable size and appearance is a major problem that presents most commercial, state, and local laboratories with a difficult challenge. However, it is important to establish whether these laboratories can follow a standard procedure and be successful in recovering and detecting these pathogenic protozoans in water samples.

Sixteen commercial laboratories were enlisted in a survey (Clancy *et al.*, 1994) to assess the ability of the laboratories to recover and detect *Giardia* and *Cryptosporidium* using the ASTM method. Filters spiked with either *Giardia* (approximately 740 cysts) and *Cryptosporidium* (approximately 500 oocysts) or with approximately 500 cells of *Oocystis minuta* (algal cells measuring 8-18 m by 5-15 m) were sent to the laboratories for analysis. Of the 11 laboratories that provided results of their analyses, four reported *O. minuta* samples as positive for either *Giardia* or *Cryptosporidium*, while four others failed to recover *Giardia* from the cyst-spiked filter and six laboratories failed to recover *Cryptosporidium* from the oocyst-spiked filter. *Giardia* cyst recovery ranged from 0.8 to 22.3% (with an average of 9.1%), while *Cryptosporidium* cyst recovery ranged from 1.3 to 5.5% (with an average of 2.8%). It was concluded that not all laboratories strictly followed the ASTM methods and that the majority of laboratories need to improve in one or more of the following areas: client response, quality of sampling equipment and directions for use, analytical methods, data accuracy, and reporting format. Expertise in microbiological identification also appeared to be lacking, as indicated by the relatively high numbers of false positives. The USEPA established an approval process for laboratories that analyzed samples for the Information Collection Rule for *Cryptosporidium* and *Giardia*. The USEPA required approved laboratories to have trained and experienced personnel performing *Cryptosporidium* and *Giardia* testing, in addition to the necessary processing equipment. Initial and continuous passing of performance evaluation samples and passing of the on-site inspection were also required.

B. Detection in Biological Samples

Diagnosis of *Cryptosporidium* infection is typically performed by examining the feces of an infected individual. Feces of patients with active cryptosporidiosis normally do not require concentration since oocysts are shed in great numbers; however, the number of oocysts can fluctuate during the course of infection (Casemore and

Roberts, 1993). This emphasizes the importance of making diagnoses using multiple specimens. Concentration methods are employed when trying to assess infection in immunocompromised patients with a history of unexplained diarrhea or in asymptomatic patients. Concentration methods including formalin-ether and formalin-ethyl acetate sedimentation are commonly used in clinical laboratories. Sheather's sucrose flotation, zinc sulfate flotation, saturated sodium chloride flotation, discontinuous Percoll gradients, and cesium chloride gradient centrifugation are methods more common in research laboratories. Evaluations of various concentration techniques have been published and the results are summarized below.

Concentration methods

A study by Bukhari and Smith (1995) comparing water-ether, sucrose density gradient, and zinc sulfate concentration methods showed significantly higher numbers of oocysts were recovered from bovine feces using water-ether concentration. Rosales *et al.* (1994) used concentration by Sheather's solution to obtain greater numbers of oocysts than by discontinuous Percoll gradients and a commercially manufactured parasite concentrator device. Concentration of oocysts from cat feces (Mtambo, 1992) was best accomplished using formalin-ether sedimentation, which recovered 37% of the original oocysts compared to 11% and 33% for zinc sulfate and sucrose flotation, respectively. Clavel *et al.* (1996b) showed that simply increasing centrifugation times augmented oocyst recovery when using the standard formalin-ether acetate concentration method. Finn *et al.* (1996) demonstrated that straining the feces contributed to the loss of oocysts. This study showed a nearly four-fold overall reduction in the number of oocysts detected using a wash procedure.

Traditional staining methods

Traditional staining methods were described in the 1994 *Cryptosporidium* Criteria Document. Numerous new staining methods and variations of traditional methods have also been employed. A review is included in Chapter 2 of the book, *Cryptosporidium* and Cryptosporidiosis (Fayer, 1997). Kang and Mathan (1996) compared five staining methods for detection of *Cryptosporidium* oocysts in fecal smears. The safranin-methylene blue technique, a modified Ziehl-Neelsen method, was used as the "gold standard" and compared to two methods each using auramine and mepacrine stains with potassium permanganate and carbol fuschin as counterstains. The authors concluded that mepacrine and auramine staining procedures were both easily performed. However, they preferred mepacrine to auramine because it is less toxic and can be used with carbol fuschin without a decolorization step. Work by Ungureanu and Pontu (1992) supported these results. In the *Cryptosporidium* Screening Guidelines established by a joint working group, Casemore and Roberts (1993) recommended an auramine method to be used as a screening method with confirmation using a modified Ziehl-Neelsen method. Acid-fast staining methods do not stain all oocysts. Entrala *et al.* (1995) showed hydrogen peroxide treatment increased the percentage of oocysts displaying acid-fast characteristics. The authors speculated that treating oocysts with hydrogen peroxide may have affected a component of the oocyst wall and composition of the oocyst contents or granules.

Immunofluorescence methods

The use of monoclonal antibody detection assays increases the sensitivity of *Cryptosporidium* detection compared to various acid-fast methods and auramine-rhodamine staining methods, as described in the following studies. Garcia *et al.* (1987) tested 297 human fecal samples using a modified acid-fast method and monoclonal

antibody staining. Sensitivity and specificity using the monoclonal antibody were 100%, while the acid-fast method failed to detect 7 of 99 positive samples. Subsequent testing by Baron *et al.* (1989), Rusnak *et al.* (1989), Garcia *et al.* (1992), Tee *et al.* (1993), Grigoriew *et al.* (1994), Alles *et al.* (1995) and Roberts *et al.* (1996) confirmed these results. Studies using monoclonal antibodies for detection of *Cryptosporidium* in animals have also shown increased sensitivity (Wee *et al.*, 1995; Mtambo *et al.*, 1992; Xiao and Herd, 1993).

EIAs

Commercial EIAs have been developed to replace time-consuming microscopic methods. Most studies have shown EIA methods perform better than (Siddons *et al.*, 1991; Dagan *et al.*, 1995) or equal to (Chapman and Rush, 1990; Rosenblatt and Sloan, 1993; Parisi and Tierno 1994) conventional microscopic methods; however, work by Newman *et al.* (1993) indicated that EIA methods were not sensitive enough to be used for patients without diarrhea. EIAs have been shown to be equal in sensitivity to the IFA (Siddons *et al.*, 1991; Rosenblatt and Sloan, 1993) and inferior using an experimental EIA (Anusz *et al.*, 1990) and a commercially available EIA (Ignatius *et al.*, 1997). Two commercial EIAs were compared to a direct immunofluorescence assay, the ProSpecT and the ColorVue (Aarnes *et al.*, 1994) and found to have differences in performance, i.e., the sensitivities were 96% and 72-76%, respectively. Specificities were 97.6-99.5% using the ProSpecT and 100% using ColorVue.

Molecular methods: PCR assays

PCR-based assays are becoming more common and may provide a useful alternative for detecting and quantifying *Cryptosporidium* in water, stools, and tissue/organ samples. Awad-El-Kariem *et al.* (1994)

described a PCR method that identifies *Cryptosporidium* at the species level and requires no DNA probes. The procedure is based on use of the published 18S rRNA genes of *C. parvum* and *C. muris*. One of the sequence 3 *MaeI* endonuclease restriction sites is present only on the *C. parvum* gene, while others are specific for *C. muris* and *C. baileyi*, which allows screening for human, mouse, and avian species, respectively. The authors suggested that the protocol they developed is adaptable to detection of small numbers of *C. parvum* oocysts in environmental samples. Morgan *et al.* (1996) noted the importance of PCR in processing clinical as well as environmental specimens suspected of being contaminated with *Cryptosporidium.* PCR primers specific for *Cryptosporidium* have been developed, and random amplified polymorphic DNA (RAPD) is a simpler approach for developing diagnostic primers, since many of the products generated by RAPD-PCR are frequently species-specific. Leng *et al.* (1996) developed an assay to identify *Cryptosporidium* DNA in bovine feces involving standardization of sample preparation and simplification of the DNA recovery process for PCR amplification and DNA-hybrid detection. The DNA recovery/PCR detection procedure can recover DNA suitable for PCR amplification and can detect 10^3 to 10^4 fewer oocysts diluted in water or buffered saline and 10^2 fewer oocysts from diarrheic fecal samples than the commercial ELISA Color-Vue-*Cryptosporidium* kit.

Amplification methods have also been described which may assist in future efforts to define the role of livestock in waterborne outbreaks of cryptosporidiosis. Blassak *et al.* (1996) described a rapid assay kit designed to test fecal samples for live *Cryptosporidium* using a gene probe method. Oocyst DNA is released by cyclical freeze/thaw and is then amplified by isothermal strand displacement using biotinylated primers. The amplification product is detected colorimetrically in a microwell system in which a complementary capture probe binds to oocyst DNA and then reacts with a horseradish peroxidase-streptavidin conjugate. This complex emits a color that can be readily detected with qualitative or semi-quantitative results. The authors suggest that

this method could be applied toward the analysis of genetic similarity among *Cryptosporidium* strains isolated from livestock and humans.

Balatbat *et al.* (1996) developed a nested PCR assay for detection of *C. parvum* directly from stool specimens. After extracting DNA from a formaldehyde-treated stool, a 400-bp fragment of DNA was amplified with two 26-mer primers. The amplicon from the first reaction was then subjected to a second round of amplification using a second set of primers. With these nested primers, a 194-bp fragment of DNA was amplified and confirmed as *C. parvum* DNA by internal probing with an enzyme-linked chemiluminescence system. The test can detect as few as 500 oocysts per gram of stool and has the potential to detect asymptomatic infections, monitor response to therapy, or monitor environmental samples. The preliminary results indicate a significantly enhanced sensitivity compared with traditional assays.

Kelly *et al.* (1995) developed a sensitive and specific PCR test to confirm *Cryptosporidium* infections. The test, which uses previously published primers to detect *Cryptosporidium* in distal duodenal biopsies, was used to identify infections in HIV-positive patients in Zambia and proved especially useful in identifying those infections that were limited to the distal small intestine.

Other methods

Serological methods have been used to monitor exposure to *Cryptosporidium.* There is limited information regarding the seroprevalence of *Cryptosporidium*-infected individuals. The serologic response to *Cryptosporidium* is discussed in section V-B of this report and by Lengerich *et al.* (1993).

Flow cytometry methods have been described for detection of *Cryptosporidium* in mice (Arrowood *et al.*, 1995). More recent studies using seeded human feces showed a four-fold increase in detection over direct immunofluorescence methods (Valdez *et al.*, 1997). Chemiluminescence assays have been used by Clavel *et al.* (1996b) to detect cultured oocysts and other fecal parasites. You *et al.* (1996a, b) showed positive detection of *Cryptosporidium* in MDCK cell cultures and the potential of this cell culture system for testing chemotherapeutic agents is promising. A reverse passive hemagglutination (RPH) assay (Farrington *et al.*, 1994) measuring agglutination of anti-oocyst antibody-coated sheep erythrocytes with oocysts in diluted fecal suspensions was compared to auramine phenol staining for detection of *Cryptosporidium* and showed equal sensitivity.

C. Water Treatment Practices

1. Introduction

Multiple barriers are used in most surface water treatment plants in an effort to prevent public exposure to waterborne pathogens like *Cryptosporidium.* These barriers include removal of pathogens from water by processes like clarification and filtration, which are generally preceded by coagulation and flocculation processes. Another type of barrier is inactivation by disinfectants like ozone and chlorine. The purpose of this section is to summarize the removal and/or inactivation of *Cryptosporidium* through multibarrier systems and through individual treatment processes. The reader is strongly encouraged to look at the referenced studies to gain detailed information regarding site-specific raw water quality and treatment conditions.

In this section, log removal is defined by the following equation:

Equation 2. $\log \text{removal} = -\log(N/N_0)$

where N is the concentration of *Cryptosporidium* oocysts remaining after treatment and N_0 is the concentration of *Cryptosporidium* oocysts prior to treatment. Log inactivation is given by a similar equation, but N and N_0 refer to the concentration of infectious *Cryptosporidium* oocysts in treated water and in untreated water, respectively. A comparison of removal efficiencies of some bench-, pilot-, and full-scale water treatment processes is found in Table 5.

2. Multibarrier Treatment

Several studies have evaluated the occurrence of *Cryptosporidium* in raw and finished waters from multibarrier treatment facilities (LeChevallier and Norton, 1995). In a survey of 72 North American drinking water plants, *Cryptosporidium* was present in 51.5% of raw water samples and in 13.4% of finished water samples. In an earlier survey of 66 drinking water plants, *Cryptosporidium* was observed in 87% and 27% of raw and finished waters, respectively. The authors attributed the different occurrence levels between studies to normal variations in raw water quality and treatment performance. The authors claimed that microscopic analyses of *Cryptosporidium* oocysts in the finished waters suggested that most of the oocysts were nonviable. However, no attempts were made to specifically assess oocyst viability in this study.

These findings suggest that a significant percentage of *Cryptosporidium* oocysts are removed by current drinking water treatment practices.

Table 5. *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 in Frey *et al.*, 1998): [a] Plummer *et al.*, 1995; [b] Patania *et al.*, 1995; [c] Schuler *et al.*, 1988; [d] Jacangelo *et al.*, 1995b; [e] Nieminski and Ongerth, 1995; [f] LeChavallier *et al.*, 1991; [g] Kelley *et al.*, 1994; [h] Anderson *et al.*, 1996; and [i] Nieminski, 1995.

Other studies have reported removal of *Cryptosporidium* oocysts through conventional filter treatments using chlorine as the primary disinfectant. The combination of coagulation, flocculation, and sedimentation achieved 3.8 log removal of oocysts in a treatment plant near Montreal, Canada (Payment and Franco, 1993). In the same treatment plant, a 4.6 log removal of oocysts was observed through coagulation, flocculation, sedimentation, and granular media filtration (also known as conventional treatment). No attempts were made to assess oocyst viability in this study.

In a more comprehensive study of a conventional treatment plant near Pittsburgh, Pennsylvania, *Cryptosporidium* oocysts were detected in 63% of raw water samples, 29% of settled water samples, and 13% of filtered water samples (States *et al*., 1997). For those cases where oocysts were detected in both raw and settled waters, the treatment plant achieved 0.8 to 1.3 log removal of oocysts prior to filtration. For those cases where oocysts were detected in both raw and filtered waters, the treatment plant achieved 1.7 to 3.6 log removal of oocysts through filtration. Oocyst viability was not measured in this study.

3. Removal of *Cryptosporidium*

Introduction

As noted in the previous section, detectable *Cryptosporidium* concentrations occur infrequently in treated waters. Because of this, the effectiveness of treatment processes has been evaluated in challenge studies where oocysts are spiked into raw water at a concentration high enough for oocysts to be detected in treated water. This section summarizes challenge study results for those processes that remove *Cryptosporidium* oocysts; challenge study results for those processes that inactivate *Cryptosporidium* oocysts are summarized in the next section.

Coagulation, flocculation, and clarification

Several clarification methods are available for drinking water treatment, and these methods are usually preceded by coagulant addition and flocculation. Currently, sedimentation is the most commonly practiced method of clarification in the United States. Other options include dissolved air flotation and sludge blanket clarification. One bench-scale study showed that dissolved air flotation was superior to sedimentation for removal of

Cryptosporidium oocysts (Plummer *et al.*, 1995). With proper coagulation and flocculation conditions, the combination of coagulation, flocculation, and dissolved air flotation could achieve 2.5 to 3.5 log removal of *Cryptosporidium.* Under similar conditions, however, the combination of coagulation, flocculation, and sedimentation could achieve no more than 1.0 log removal of oocysts. Similar bench-scale studies showed that a 1.3 to 2.8 log removal of *Cryptosporidium* could be achieved by the combination of coagulation, flocculation, and dissolved air flotation (Hall *et al.*, 1995).

Plummer *et al.* (1995) observed relatively weak correlations between the log removal of *Cryptosporidium* and removal of turbidity ($r^2 = 0.53$), UV absorbance ($r^2 = 0.52$), or dissolved organic carbon ($r^2 = 0.50$).

Coagulation, flocculation, sedimentation, and filtration (conventional treatment)

Pilot-scale treatment studies with two water supplies in the western United States showed that conventional treatment could obtain 3.0 to 6.2 log removal of *Cryptosporidium*, with a median of approximately 4.6 log removal (Patania *et al.*, 1995). An average of 3.0 log removal was observed in another pilot-scale study in Utah, with a range of 1.9 to 4.0 log removal (Nieminski and Ongerth, 1995). Another Utah study was designed to evaluate *Cryptosporidium* removal in a full-scale treatment plant that was not delivering water to customers. In this case, removal efficiencies ranged from 1.9 to 2.8 log removal with an average of 2.3 log (Nieminski and Ongerth, 1995). Actual performance depends on numerous factors including source water quality, chemical pretreatment conditions (e.g., coagulant dose and pH), filtration rate, bed depth, and filter media types. As noted earlier, the reader is advised to read the referenced reports for more detailed information regarding the results summarized here.

The results to date suggest that the 2.0 log removal credit proposed for *Cryptosporidium* removal by conventional treatment is reasonable and, as shown by the above data, may be conservative. However, studies have only been published for waters from the western United States and need to be confirmed with results from other types of water supplies from other geographic areas. As noted in Section III.B.1, any watershed, river, or reservoir is subject to a complex set of watershed characteristics and watershed processes (Crockett and Haas, 1997; LeChevallier *et al.*, 1997; States *et al.*, 1997).

After combining data from the two Utah studies, Nieminski and Ongerth (1995) reported an r^2 of 0.79 for the relationship between log removal of *Cryptosporidium* and log removal of 4-7 m sized particles. The correlation between log removal of *Cryptosporidium* and log removal of turbidity was weaker, with an r^2 of 0.55. Patania *et al*. (1995) reported no significant correlations between log removal of *Cryptosporidium* and log removals of turbidity, 1-2 m sized particles, 2-5 m sized particles, 5-15 m sized particles. These latter results were obtained from a broader range of source waters and suggest that correlations between *Cryptosporidium* removal and removal of surrogate indicators may be site specific. Further work is necessary before definitive conclusions can be reached. These two studies also based their conclusions on data obtained from different types of filtration practices (e.g., conventional treatment, direct filtration, in-line filtration). Further analysis is needed to determine whether their observations were dependent on the type of filtration practice.

Although no significant correlations were obtained between *Cryptosporidium* removal and turbidity removal, Patania *et al*. (1995) observed that filter effluent turbidities less than or equal to 0.1 NTU were required to

obtain a 5 log *Cryptosporidium* removal on a reliable basis. Filter effluent turbidities of 0.2 NTU or less were needed to reliably maintain a 4 log *Cryptosporidium* removal.

Coagulation, flocculation, flotation, and filtration

The effect of substituting sedimentation with dissolved air flotation on filtered water *Cryptosporidium* concentrations has not been directly compared in literature reports. However, pilot-scale studies in the United Kingdom have shown that the combination of coagulation, flocculation, flotation, and filtration can achieve 2.9 to 4.4 log removal of oocysts (Hall *et al*., 1995). These results are consistent with those described above.

Coagulation, flocculation, and filtration (direct filtration)

The performance of direct filtration was assessed for the same Utah waters described in the conventional treatment section (Nieminski and Ongerth, 1995). For the pilot-scale system, *Cryptosporidium* removal efficiencies ranged from 1.3 to 3.6 log with an average of 3.0 log. The full-scale system achieved an average oocyst removal of 2.8 log with a range of 2.6 to 2.9 log. Comparable removals were observed in pilot-scale studies with water from Seattle (Ongerth and Pecoraro, 1995). Results in this study showed 2.7 to 3.1 log removal of *Cryptosporidium.*

Direct comparisons between direct and conventional filtration were only obtained with the pilot-scale system in Utah. Results indicate that there was no statistically significant difference between the two types of filtration. At this time, this conclusion can only be applied to this case and further studies are necessary to determine those situations in which direct filtration achieves performance comparable to conventional treatment.

Coagulation and filtration (in-line filtration)

Using water from the Seattle water supply, pilot-scale treatment studies showed that in-line filtration could obtain 1.6 to 4.2 log removal of *Cryptosporidium.* The median value was approximately 2.8 log removal (Patania *et al*., 1995). Unfortunately, a direct comparison between in-line filtration and conventional treatment has not been made for the same water supply.

Diatomaceous earth filtration

A recent study with water from Sydney, Australia, concluded that diatomaceous earth filtration could perform significantly better than conventional treatment for removal of *Cryptosporidium* oocysts (Ongerth and Hutton, 1997). In this bench-scale study, removal efficiencies ranged from 3.6 to 6.7 log. Although these results appear to show superior performance of diatomaceous earth filtration, they were performed at filtration rates lower than those commonly used in conventional treatment facilities. Another study has shown comparable *Cryptosporidium* oocyst removal by pilot-scale diatomaceous earth filtration with a Pennsylvania water sample, in which removal efficiencies ranged from 4.6 to 5.9 log (Schuler *et al*., 1991).

Slow sand filtration

Several studies have been performed to evaluate *Cryptosporidium* oocyst removal by slow sand filtration. Removal efficiencies ranging from 3.9 to 7.1 log were observed in pilot-scale studies with water from Pennsylvania (Schuler *et al*., 1991). Another study in the United Kingdom showed that oocyst removal exceeded 4.5 log removal (Timms *et al*., 1995). Only 0.3 log oocyst removal was obtained in a slow sand filter located in British Columbia, Canada. However, the filter media in this filter did not meet standard

specifications for slow sand filters (Fogel *et al*., 1993). Therefore, the overall results obtained to date suggest that slow sand filtration is an effective barrier to oocyst passage when the process is properly designed.

Membrane processes

In several microfiltration and ultrafiltration experiments, *Cryptosporidium* oocysts were not detected in treated waters when the membranes were intact (Jacangelo *et al*., 1995a). This result was observed with feed oocyst concentrations as high as 9.1×10^4 oocysts/L, suggesting a removal capability of more than 7.1 log. This result is expected because the pores within the membrane skin are smaller than *Cryptosporidium* oocysts. More recent studies have shown that *Cryptosporidium* oocysts could pass through a ceramic microfiltration membrane having a nominal porosity of 0.2 m (Drozd and Schwartzbrod, 1997). In this case, oocyst removals ranged from 4.3 to 5.5 log. However, no attempts were made to assess the integrity of the membrane and its associated equipment in this study.

4. Inactivation of *Cryptosporidium*

Introduction

The majority of studies performed to date have evaluated *Cryptosporidium* inactivation in buffered, demand-free waters and in small, batch reactors. Very little is known about the ability of alternative disinfectants to inactivate *Cryptosporidium* oocysts in natural waters or in flow-through treatment systems. Results depend on the method used to quantify inactivation and, if mouse infectivity is used to quantify inactivation, results may depend on the strain of mouse and on the strain of *Cryptosporidium* used in the study. For these reasons, the reader is cautioned against extrapolating information presented in this section to inactivation of

Cryptosporidium in natural waters and to infectivity in humans. The purpose of this section is to summarize the inactivation work performed to date for four disinfectant chemicals – ozone, chlorine dioxide, chlorine, and monochloramine.

Ozone

Cryptosporidium inactivation by ozone has received a significant amount of attention. Ozone clearly achieves the most significant levels of *Cryptosporidium* inactivation when compared to the other three disinfectants listed above (Korich *et al*., 1990; Finch *et al*., 1997). In order to achieve 3 log inactivation of *Cryptosporidium* at pH 7, the product of contact time and ozone concentration (CT) needs to be in the range of 8 to 16 mg•min/L at 7°C and in the range of 3 to 15 mg•min/L at 22°C (Finch *et al*., 1993). CT values for a 2 log inactivation of *Cryptosporidium* by ozone at pH 7 are in the 5 to 10 mg•min/L range at 7°C and in the 2 to 8 mg•min/L range at 22°C. It is important to stress that these values are based on mean performance and do not account for the wide variability observed in test results. Overall results observed by other investigators appear to be in general agreement, when the variability in data is taken into account (Peeters *et al*., 1989; Korich *et al*., 1990; Parker *et al*., 1993; Owens *et al*., 1994a and b; Quinn *et al*., 1996; Finch *et al*., 1997).

Chlorine dioxide

Results obtained to date suggest that chlorine dioxide is the second most effective disinfectant on the above list (Peeters *et al*., 1989; Korich *et al*., 1990; Finch *et al*., 1997, Liyanage *et al*., 1997a). CT values needed for *Cryptosporidium* inactivation by chlorine dioxide are considerably higher than those needed for ozone. To achieve a 1 log inactivation of *Cryptosporidium* at pH 7 and 25°C, CT values of approximately 60 mg•min/L

would be necessary. At pH 8, the required CT values appear to be greater than this. Recent results have shown that chlorine dioxide may be more effective if ozone is applied upstream of the chlorine dioxide addition point (Liyanage *et al*., 1997b). However, this practice is not likely to become common in the United States due to the implementation of the Stage I Disinfectants and Disinfection Byproducts Rule, which set a maximum residual disinfectant level goal (MRDLG) for chlorine dioxide of 0.8 mg/L.

Chlorine

At CT values commonly used in drinking water treatment, chlorine does not achieve more than a 1 log inactivation of *Cryptosporidium* oocysts (Korich *et al*., 1990; Fayer, 1995; Finch *et al*., 1997; Gyurek *et al*., 1997; Venczel *et al*., 1997). This is true even in demand-free water at pH 6 and 22°C, conditions under which chlorine can be expected to achieve the best level of performance. Recent screening studies have suggested that the ability of chlorine to inactivate *Cryptosporidium* may be enhanced by preozonation (Finch *et al*., 1997). At this time, published reports are very preliminary and further studies will be needed to determine if this phenomenon is observed in natural waters and in other laboratories.

Monochloramine

Ever since the passage of the Surface Water Treatment Rule, monochloramine has not been used very frequently as a primary disinfectant in drinking water treatment. Like chlorine, monochloramine is not capable of achieving more than a 1 log inactivation of *Cryptosporidium* at CT values commonly encountered in treatment practice (Korich *et al*., 1990; Finch *et al*., 1997; Gyurek *et al*., 1997). Also, preliminary studies have shown that the ability of monochloramine to inactivate *Cryptosporidium* may be enhanced by preozonation or by

prechlorination (Finch *et al*., 1997; Gyurek *et al*., 1997). Again, published reports are very preliminary and further studies will be needed to determine if this phenomenon is observed in natural waters and in other laboratories.

Mixed oxidants

A proprietary system that electrochemically produces a mixed oxidant solution was recently evaluated for its ability to inactivate *Cryptosporidium* oocysts (Venczel *et al*., 1997). This system was observed to achieve more than a 3 log inactivation of *Cryptosporidium* oocysts with a 5 mg/L oxidant dose and a 4-hour contact time.

UV irradiation

Recent studies have demonstrated that some ultraviolet irradiation technologies may be promising for *Cryptosporidium* inactivation (Campbell *et al*., 1995; Arrowood *et al*., 1996). Because the technologies employed in these two studies are not comparable, their results cannot be compared. In one case, ultraviolet irradiation produced a 2 to 3 log inactivation of *Cryptosporidium* oocysts at ultraviolet doses and contact times achievable by commercial equipment (Campbell *et al*., 1995). In the other study, up to 6 log inactivation was observed with an altemative piece of commercial equipment (Arrowood *et al*., 1996).

D. Summary

Analysis of water samples

USEPA Method 1622 uses a capsule filter to collect *Cryptosporidium* oocysts from water samples. Other collection methods include the use of filters of varying compositions (e.g., wound yarn, cellulose acetate). Capsule filters and cellulose acetate membrane filters appear to have better performance than wound yarn filters. Calcium carbonate flocculation methods, which can concentrate up to 10 L of water, have also been shown to be superior to wound yarn 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 ASTM ICR methods; however, the methods still require interlaboratory validation. Immunomagnetic capture and flow cytometry also show considerable recovery increases using either seeded or environmental samples. Immunomagnetic capture is the currently recommended method for recovering oocysts from water samples, as described in Method 1622. Laser scanning devices have also performed well in early studies, but more research is required. Several applications of PCR for the detection of *Cryptosporidium* have been described in the literature, some of which may be able to distinguish viable from nonviable oocysts; however, enzymatic inhibition in PCR assays remains problematic.

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 have produced conservative estimates of oocyst viability compared to animal modeling data; however, limitations in viability assays have precluded their routine use in environmental samples. The USEPA has established an approval process for laboratories performing detection of *Cryptosporidium* and *Giardia* in water.

Analysis of biological samples

The 1994 *Cryptosporidium* Criteria Document described the increased sensitivity of IFA-based procedures. Traditional staining methods such as the Ziehl-Neelsen stain, however, are still widely used. EIA methods are fast, inexpensive, easily performed, and show sensitivity approaching that of IFA methods. However, a lack of confirmatory analyses may preclude the routine use of EIA methods. Enzyme immunoassays may be useful for busy hospital laboratories or large-scale screening surveys. Several PCR-based methods capable of distinguishing differences among specific strains have been described in the literature. As with testing the efficacy of different water analysis methods, interlaboratory comparisons require strict adherence to oocyst quality and rigorous enumeration procedures. Recommendations by Klonicki *et al.* (1997) should be observed in future studies.

Summary of removal studies

Of the technologies available to the drinking water industry, membrane processes appear to provide the most significant levels of *Cryptosporidium* removal. However, full-scale testing of membrane processes has not yet been conducted. Conventional treatment practices appear capable of meeting at least 2 log removal in most of the cases studied to date. Although direct filtration and in-line filtration appear to be less effective than conventional treatment, this has not been demonstrated in a conclusive manner for full-scale treatment systems. In bench- and pilot-scale studies, alternative technologies like diatomaceous earth filtration and slow sand filtration appear capable of achieving comparable, if not better, levels of *Cryptosporidium* removal than conventional treatment.

Summary of inactivation studies

Ozone appears to be the best chemical disinfectant for *Cryptosporidium* inactivation at this time. The mixed oxidant and ultraviolet light systems appear to be promising but have only been tested in minimal fashion compared with ozone. Also holding some promise are the sequential disinfection systems of ozone followed by the combination of chlorine and ozone, followed by monochloramine. Very few studies have evaluated *Cryptosporidium* inactivation in natural waters.

VIII. 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.

Many of the data gaps in our knowledge regarding *Cryptosporidium* previously identified in the 1994 *Cryptosporidium* Criteria Document have been filled, and an enormous amount of information has become available from research conducted in association with the Information Collection Rule. Data gaps that persist in the areas of source water occurrence, health effects, risk assessment, analysis, and treatment are described below.

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: Progress has been made in identifying compounds that can be used for human and animal therapy/treatment, although evaluation, validation, and clinical trials will be required, and these drugs will be subject to FDA approval after such research and clinical trials are completed. However, studies to develop new drugs should be continued. Information about the mechanism of pathogenicity might explain strain differences in the production of diarrhea. There has been very little progress in elucidating the pathogenic mechanisms involved in cryptosporidiosis, but USEPA-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 determine 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, such as the work done by Nahrstedt and Gimbel (1996) and Teunis and Havelaar (1999) described in Section VI.

Analysis: Research efforts for recovery of *Cryptosporidium* oocysts from water samples as well as from clinical samples have been improved and many of the steps in these processes that historically have been responsible for oocyst loss have been identified. Studies comparing methods have been conducted, and the advantages and disadvantages of various approaches have been elucidated. New detection methods are being developed, especially those using molecular biology approaches (e.g., PCR/gene probe procedures), laser-based technologies, and computer-assisted microscopy. Using these approaches, methods for determination of oocyst survivability in the environment and infectivity should improve significantly. Detection methods continue to be

quite variable and the need for a standard method that is accurate, precise, quick, and affordable still exists. Many of the newer technologies are yet unproven with real-world samples, and validation testing must be completed. The analysis of large sample volumes still presents a challenge for detection of *Cryptosporidium* using routine collection methods. 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.

Treatment: There is a great need to develop, identify, and evaluate new 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 efficiency 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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