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CRYPTOSPORIDIUM AND *GIARDIA* OCCURRENCE ASSESSMENT FOR THE INTERIM ENHANCED SURFACE WATER TREATMENT RULE

CRYPTOSPORIDIUM AND *GIARDIA* OCCURRENCE ASSESSMENT FOR THE INTERIM ENHANCED SURFACE WATER TREATMENT RULE

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EXECUTIVE SUMMARY

The 1996 Amendments to the Safe Drinking Water Act require EPA to promulgate the Interim Enhanced Surface Water Treatment Rule (IESWTR) and the Stage 1 Disinfectants and Disinfection Byproducts Rule (D/DBPR) by November 1998 (Section 1412 (b)(2)(C)). EPA proposed these two regulations, IESWTR (59 FR 38832) and D/DBPR (59 FR 38668), on July 29, 1994. The IESWTR was developed to provide additional microbial protection beyond that which is prescribed in the Surface Water Treatment Rule (54 FR 27486; June 29, 1989) and to control for *Cryptosporidium*. The D/DBPR was developed to limit the levels of several disinfectants and disinfection byproducts resulting from their use. EPA believes that the two rules need to be promulgated simultaneously to assure concurrent compliance and a balanced risk-based implementation. The IESWTR pertains to public water systems serving 10,000 people or greater and the D/DBPR applies to community water systems and non-transient non-community water systems that treat their water with a chemical disinfectant for either primary or residual treatment.

The Agency has requested public comment regarding these rules on three occasions and has engaged in several stakeholder meetings to discuss and share information pertaining to rule development. In February 1997, EPA established the Microbial and D/DBP Advisory Committee under the Federal Advisory Committee Act (FACA) to collect, share and analyze new information and data, as well as to build a consensus relating to the regulatory implications of this new information. As a result of these negotiations, the Agency published Notice of Data Availability (NODA) for the IESWTR (59 FR 59486) and the D/DBPR (62 FR 59388) in November 1997. An additional NODA for the D/DBPR was published in March 1998 which included new information and analyses that became available after the 1997 NODA (63 FR 15674).

The following document, "*Cryptosporidium* and *Giardia* Occurrence Assessment", was developed to support the IESWTR. The intent of the document is to provide available information on the occurrence of *Cryptosporidium* and *Giardia* in surface water as well as finished water supplies. The document provides information on: the characteristics of *Cryptosporidium* and *Giardia*, occurrence of *Cryptosporidium* and *Giardia*; analytical methodologies utilized to measure the contaminants; distribution of the contaminants in source water and finished water; and populations potentially exposed to the contaminants. The document emphasizes the occurrence of *Cryptosporidium* and *Giardia* because they are persistent in the water environment, and small doses are able to cause infection in humans.

The occurrence of *Cryptosporidium* and *Giardia* in water intended for drinking has been documented in a range of different instances for source water, finished water, and ground water. This document presents information from surveillance reports regarding occurrence of *Cryptosporidium* and *Giardia* in drinking water. The Centers for Disease Control and Prevention (CDC) reports indicate that infections by these organisms have caused over 400,000 persons in the United States to become ill since 1991. Over 50 immunocompromised individuals have died after contracting cryptosporidiosis during waterborne disease outbreaks since 1991.

To develop this document literature searches were conducted using several research databases. EPA notes that the occurrence data compiled in this document and the current national estimate of

Cryptosporidium and *Giardia* occurrence reflects existing data. Substantial additional information that will be used to strengthen an estimate of national occurrence of *Cryptosporidium* and *Giardia* is currently being collected under the Information Collection Rule (ICR). However, EPA believes that the information in this document is sufficient to conclude that *Cryptosporidium* and *Giardia* can and do occur in public water supplies at levels which may pose a risk to human health.

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ABBREVIATIONS

AGI	acute gastrointestinal illness of unknown etiology
AIDS	acquired immunodeficiency syndrome
ASTM	American Society for Testing and Materials
AWWA	American Water Works Association
AWWARF	American Water Works Association Research Foundation
AWWSC	American Water Works Service Company
BAT	Best Available Technology
CDC	U.S. Centers for Disease Control and Prevention
CPE	cytopathic effect
CSO	combined sewer overflow
D/DBP	disinfectants/disinfection byproduct
DAF	dissolved-air flotation
DAPI	4',6-diamidino-2-phenylindole
DE	diatomaceous earth
DIC	differential interference contrast
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
ESWTR	Enhanced Surface Water Treatment Rule
FACA	Federal Advisory Committee Act
FALS	forward-angle light scatter
GWUDI	Groundwater Under the Direct Influence of Surface Water
HAV	hepatitis A virus
HIV	human immunodeficiency virus
ICR	Information Collection Rule
IESWTR	Interim Enhanced Surface Water Treatment Rule
IFA	indirect fluorescent antibody
IMS	immunomagnetic separation
LO	light obscuration
LTESWTR	Long-term Enhanced Surface Water Treatment Rule
M/DBP	microbial/disinfection byproduct
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	microfiltration
MPA	microscopic particulate analysis
MPN	Most Probable Number
MRDL	maximum residual disinfectant level
MRDLG	maximum residual disinfectant level goal
NF	nanofiltration
NODA	Notice of Data Availability
NTU	nepholometric turbidity unit
OGWDW	Office of Ground Water and Drinking Water
OST	Office of Science and Technology
PCR	polymerase chain reaction
PE	performance evaluation
PI	propidium iodide
PWS	public water system

QC	quality control
RNA	ribonucleic acid
RO	reverse osmosis
rRNA	ribosomal ribonucleic acid
SDWA	Safe Drinking Water Act
SEM	scanning electron microscopy
SNWS	Southern Nevada Water System
SRSV	small round structured virus
SWTR	Surface Water Treatment Rule
TTHM	total trihalomethane
TWG	Technology Working Group
UF	ultrafiltration
USDA	U.S. Department of Agriculture

1. INTRODUCTION

The U.S. Environmental Protection Agency's (EPA) Office of Ground Water and Drinking Water (OGWDW) is developing a set of interrelated drinking water regulations to control disinfectants and disinfection byproducts (D/DBPs) and to refine control of microbiological pathogens in public water systems. The control of microbiological contaminants is complicated because commonly used disinfection processes themselves may pose serious health risks. The EPA, under direction from Congress, is planning to supplement the existing 1989 Surface Water Treatment Rule (SWTR) with the Enhanced Surface Water Treatment Rule (ESWTR) to further guard against waterborne disease transmission through water supply systems. The ESWTR (divided into two phases, "interim" and "long-term") will revise and strengthen the existing SWTR, which presently contains filtration and disinfection requirements for surface water systems and groundwater supplies under the direct influence (GWUDI) of surface water. Removal or inactivation of *Giardia*, viruses, and bacteria from source water is required. To address these complex issues, EPA proposed the National Primary Drinking Water Regulations: Interim Enhanced Surface Water Treatment Rule on July 29, 1994.

This document contains information on the analytical methods, occurrence, distribution, and populations potentially exposed to the contaminants of concern. It also includes information related to the turbidity provisions recommended by the Microbial and D/DBP Advisory Committee. The data on occurrence and exposure support several aspects of the drinking water regulatory development process:

- Contaminant occurrence levels in public water supplies, of various source and size characteristics, provide EPA with the basis for estimating the number of systems and the size of the affected populations currently experiencing contaminant levels exceeding the treatment alternatives under consideration.
- Contaminant occurrence levels in the distribution systems of public water supplies of various source and size characteristics will be used in conducting the cost impact analyses of the regulatory alternatives.
- Contaminant occurrence levels will support the identification of Best Available Technology (BAT) necessary for setting the MCL and for granting variances.
- Occurrence data will be used to develop exposure assessments and, subsequently, the contribution of drinking water, relative to other sources of exposure, to total intake for setting the MCLG for contaminants.

1.1 REGULATORY BACKGROUND

On June 29, 1989, EPA promulgated the SWTR (54 *FR* 27486) that included criteria for disinfection and filtration requirements and procedures by which the states are allowed to determine which systems must install filtration. In 1990, an independent panel, established by Congress, cited drinking water contamination as one of the highest ranking environmental risks (SAB 1990). In response, EPA revisited the control of microbiological contaminants in drinking water supplies. The following is a brief chronology of EPA's

rulemaking activities on microbiological drinking water contaminants, starting with promulgation of the SWTR:

- *1989*—The SWTR is promulgated
- *1992*—A negotiated rulemaking is initiated
- *1994*—Three rules are proposed: Stage 1 D/DBP Rule, the Interim ESWTR (IESWTR), and the Information Collection Rule (ICR)
- *1996*—ICR is promulgated
- *1996*—The Safe Drinking Water Act (SDWA) is reauthorized
- *1997*—Microbial and D/DBP Advisory Committee is established
- *1998*—IESWTR will be promulgated.

Each of these milestones leading up to promulgation of the IESWTR is summarized below.

1.1.1 Surface Water Treatment Rule

The SWTR set maximum contaminant level goals (MCLGs) of zero for *Giardia lamblia*, viruses, and *Legionella* and promulgated national primary drinking water regulations for public water systems using surface water sources or groundwater supplies under the direct influence of surface water. The SWTR includes: criteria under which filtration (including coagulation and sedimentation, as appropriate) are required; procedures by which the states are allowed to determine which systems must install filtration; and disinfection requirements. The filtration and disinfection requirements are treatment technique requirements to protect against the adverse health effects of exposure to *Giardia lamblia*, viruses, *Legionella*, and heterotrophic bacteria, as well as many other pathogenic organisms that are removed by these treatment techniques. The SWTR also requires public water systems (PWSs) that use conventional treatment or direct filtration to achieve a turbidity performance criterion of 0.5 NTU for 95 percent of the time over a 1-month period based on 4-hour sampling intervals. Under this rule, systems may not exceed a maximum turbidity of 5 NTU at any time. Also, systems are required to achieve a 3-log reduction in *Giardia lamblia* cysts and 4-log reduction in viruses through a combination of filtration and disinfection.

EPA promulgated the SWTR to control pathogens in surface water. The goal of the SWTR is to reduce risk to less than one infection per year per 10,000 people (10^{-4}). The SWTR, however, may have several shortcomings. One potential shortcoming is that the source waters of some PWSs have a high pathogen concentration that, when reduced by levels required under the SWTR, still may not meet a common health goal that would apply to all systems. Another apparent deficiency is that the SWTR does not specifically address *Cryptosporidium*.

Systems could overcome shortcomings of the SWTR by increasing the disinfectant dose for greater pathogen inactivation. However, increasing existing disinfection levels does not appear to be an effective strategy for controlling *Cryptosporidium* because the oocysts have been shown to be resistant to disinfection. Moreover, disease-causing organisms (i.e., pathogens) are not the only health threat in drinking water. The disinfectants used to control pathogens may produce toxic or carcinogenic disinfection byproducts (DBPs) when they react with chemicals in the water. As such, an important question facing water supply professionals is how to minimize the health risk from both pathogens and DBPs simultaneously.

Under the current SWTR, EPA regulates turbidity to ensure treatment effectiveness. The most important reason for monitoring turbidity is to assess the performance of the filtration process (including coagulation, flocculation, and sedimentation). As filtered water turbidities are lowered, better pathogen removal tends to occur as a function of improved filtration performance. A high turbidity level (whether a short-term spike or one longer lasting) suggests a deficiency in the filtration process that could potentially result in the introduction of pathogens into the distribution system. Moreover, the consequences associated with the loss of the filtration barrier, even for a short time, might be especially severe if other barriers to prevent the introduction of fecal contamination into the distribution system (e.g., watershed control) are inadequate.

1.1.2 Negotiated Rulemaking

In 1992, EPA initiated a negotiated rulemaking to develop a D/DBP Rule. The negotiators included representatives of state and local health and regulatory agencies, public water systems, elected officials, consumer groups, and environmental groups. The Negotiating Committee recognized that existing risks from D/DBPs could be large. The Committee also emphasized that improved control of DBPs must not come at the expense of reduced microbial protection. This compromise is commonly referred to as the "risk-risk" tradeoff. Utilities that make changes in existing treatment, to comply with the new regulations for DBPs, should not impose any significant increases in risk of exposure to microbial pathogens.

Early in the process, the Negotiating Committee agreed that large amounts of information necessary to understand how to optimize the use of disinfectants to concurrently minimize microbial and D/DBP risk on a plant-specific basis were unavailable. Nevertheless, the Negotiating Committee agreed to propose a D/DBP rule to extend coverage to all community and nontransient noncommunity water systems that use disinfectants. This rule proposed to reduce the current total trihalomethane (TTHM) maximum contaminant level (MCL), regulate additional disinfection byproducts, set limits for the use of disinfectants, and reduce the level of organic compounds in the source water that may react with disinfectants to form byproducts.

The Negotiating Committee agreed to develop three sets of rules: a two-staged D/DBP Rule (59 *FR* 38668; July 29, 1994), an IESWTR (59 *FR* 38832; July 29, 1994), and an ICR (59 *FR* 6332; February 10, 1994).

The Negotiating Committee agreed that a "long-term" ESWTR (LTESWTR) would be needed for systems serving fewer than 10,000 people when the results of more research and water quality monitoring became available. The LTESWTR could also include additional refinements for larger systems.

1.1.5 The Information Collection Rule

The Negotiating Committee decided that to develop a reasonable set of rules and to understand more fully the limitations of the current SWTR, additional field data were critical. A key component of the regulatory negotiation agreement was the promulgation of the ICR. The ICR is a monitoring and data reporting rule that was promulgated on May 14, 1996 (61 *FR* 24354). The purpose of the ICR is to collect occurrence and treatment information to evaluate the need for possible changes to the current SWTR and existing microbial treatment practices and to evaluate the need for future regulation for D/DBPs. The ICR will provide EPA with information on the national occurrence in drinking water of chemical byproducts (formed when disinfectants used for microbial control react with organic compounds already present in source water) and disease-causing microorganisms. Engineering data on how PWSs currently control chemical and microbial contaminants will also be collected under the ICR. This information will be used to assess the potential health problems created by the presence of DBPs and pathogens in drinking water and the extent and severity of risk to assist in developing regulatory and public health decisions. The ICR data will be used for developing the LTESWTR and the Stage 2 D/DBP Rule.

1.1.6 1996 Safe Drinking Water Act Reauthorization Implications

In 1996, Congress reauthorized the SDWA. Language included in the 1996 SDWA Amendments addresses provisions related to the SWTR and the ESWTR. Of note, as part of the 1996 SDWA Amendments, Congress established a deadline of November 1998 for the promulgation of both the Stage 1 D/DBP Rule and IESWTR. The amendments also established a deadline of May 2002 for the final Stage 2 D/DBP and November 2000 for the LTESWTR. Drinking water plant design information obtained under the ICR, as well as data and information from other sources, will be used by EPA to develop, analyze, and assess options for proceeding with the Stage 1 D/DBP Rule and IESWTR.

1.1.7 Microbial and D/DBP Advisory Committee

In May 1996, EPA initiated a series of public information meetings to exchange information on issues related to the development of the IESWTR and the Stage 1 D/DBP Rule. The EPA established the Microbial and D/DBP Advisory Committee under the Federal Advisory Committee Act (FACA) on February 12, 1997, to collect, share, and analyze new information and data, as well as to build consensus on the regulatory implications of this new information. The Committee consists of 20 members representing EPA, state and local public health and regulatory agencies, local elected officials, drinking water suppliers, chemical and equipment manufacturers, and public interest groups.

1.2 PURPOSE OF THE OCCURRENCE DOCUMENT

The purpose of this occurrence document is to support the IESWTR regulatory development process by summarizing currently available information on the occurrence of *Cryptosporidium* and *Giardia* in drinking water. This document emphasizes *Cryptosporidium* and *Giardia* because they are important waterborne pathogens that can survive for months in the water environment, are very resistant to water disinfection, and can cause infection from a small number of oocysts or cysts. Additionally, humans, as well as domestic or wild animals, can serve as reservoirs and important primary or intermediate sources of infection, particularly via surface water contamination.

To provide preliminary support to the above regulatory processes, an effort to collect, review, and summarize occurrence data was undertaken. This document presents the results of that effort.

Information collection began with computer-based literature searches conducted by EPA and EPA's contractor to identify relevant articles and studies from the scientific literature. The articles and studies were identified using key drinking water terms, contaminant names, or authors' names. The literature searches were performed on several bibliographic services to ensure the most available data were identified.

Over 400 articles, reports, and studies were identified and reviewed for possible relevance to the topics of interest. Relevant information in these articles was identified and summarized for inclusion in this document. When authors presented conclusions, their conclusions were included in this document.

1.3 DOCUMENT ORGANIZATION

This document is organized into eight chapters and two appendices. A description of each of the remaining sections is presented below.

- ***Chapter 2—Characteristics of Pathogens of Concern:*** In this section, the biological characteristics of *Cryptosporidium* and *Giardia* are described as a foundation for understanding the risks of disease transmission.

- ***Chapter 3—Detection and Enumeration of Pathogens:*** In this section, the analytical method approved by EPA for the ICR to detect and enumerate *Cryptosporidium* oocysts in water and the advantages and limitations of the method are described. The ICR method is discussed in this document because it is probable that most data discussed in this document were generated via this method.

- ***Chapter 4—Sources and Transmission of Pathogens:*** This section provides an overview of the waterborne and direct contact transmission of *Cryptosporidium* and, to a lesser extent, *Giardia*, including sources of the parasites in the environment and observed efficiencies of water filtration systems.

- ***Chapter 5—Occurrence of Pathogens in Drinking Water:*** Chapter 5 presents information on recent disease outbreaks, as well as monitoring and survey data providing evidence of the

occurrence of pathogens in drinking water derived from either surface water or groundwater under the direct influence (GWUDI) of surface water.

- ***Chapter 6—Occurrence of Elevated Turbidity in Finished Water:*** Chapter 6 summarizes occurrence data for finished water turbidity, a parameter regulated in the existing SWTR to ensure treatment effectiveness.

- ***Chapter 7—Population Profile:*** Chapter 7 describes the populations at potential risk from exposure to waterborne *Cryptosporidium* and *Giardia*.

- ***Chapter 8—References cited.***

- ***Appendix A—Pathogen Detection Methods:*** Appendix A reviews alternative analytical methods for *Giardia* and *Cryptosporidium*.

2. CHARACTERISTICS OF PATHOGENS OF CONCERN

This chapter summarizes the characteristics of two waterborne disease-causing organisms (*Giardia* and *Cryptosporidium*), the control of which in drinking water is the focus of the turbidity provisions recommended by the Advisory Committee. Because *Giardia* and, in particular, *Cryptosporidium*, are the focus of the interim rule, their occurrence in drinking water is the main subject of this report. Other waterborne pathogens are not described. Section 2.1 describes the parasite *Cryptosporidium*, its taxonomy, host range, life cycle, fate and transport in environmental media, and environmental persistence and viability (of the oocyst, the infective stage of the organism). Section 2.2 addresses these properties for *Giardia*.

2.1 *CRYPTOSPORIDIUM* AND *CRYPTOSPORIDIUM PARVUM*

This section presents a summary of currently available information on *Cryptosporidium*, *Cryptosporidium parvum*, and other species. Observations on the persistence of the infective oocyst stage under a range of environmental conditions are discussed. Biological and epidemiological factors contributing to the potential for waterborne transmission of cryptosporidiosis include the following (adapted from Casemore 1990):

- A wide mammalian host range, including wild and domesticated animals
- Life cycle completed in a single host species
- Oocysts excreted in large numbers (enhanced by an auto-infective cycle)
- Oocysts excreted fully infective (no external "ripening" required)
- Oocysts resistant to most environmental extremes and to common disinfectants
- Probable low infective dose (DuPont et al. 1995)
- Endemic disease with excretion of oocysts by asymptomatic carriers
- Long-term excretion of oocysts by animals and chronically ill patients (e.g., immuno-compromised individuals)
- Lack of effective medical treatment
- Secondary, direct transmission routes (e.g., among diapered children in day care settings)
- Ubiquitous geographic distribution
- Lack of specific and fully efficacious treatment

2.1.1 Taxonomy

Members of the genus *Cryptosporidium* are taxonomically classified in the phylum Apicomplexa, order Eucoccidiorida, suborder Eimeriorina, and family Cryptosporidiidae (Fayer et al. 1997b, O'Donoghue 1995). All members of the Apicomplexa are parasitic, with some of them extremely important as disease agents (Levine 1985). Within the Apicomplexa are several related genera referred to collectively as coccidia. The majority of coccidia are small protozoa that complete their life cycles intracellularly, that is, within the digestive tract epithelium, liver, kidney, blood cells, or other tissues of the host. *Cryptosporidium* species infect epithelial surfaces, particularly those of the intestines. These species can be found in a wide range of vertebrates, including humans (Fayer et al. 1997b).

There is some uncertainty about the taxonomy (i.e., classification) of species within the genus *Cryptosporidium*. Until 1980, classification was based on the assumption that a particular species only infected one type of animal (i.e., each host species harbored a separate species of *Cryptosporidium*) (Fayer and Ungar 1986). The "single host/single species" assumption has been shown to be incorrect. Evidence has been accumulating since 1980 to suggest that species specificity is not a characteristic shared by all or even most isolates of *Cryptosporidium* (Tzipori 1985). Hence, other more appropriate taxonomy schemes have been suggested. Of the original 21 different species described, most are now considered invalid (Tzipori and Griffiths 1998). O'Donoghue (1995) lists six *Cryptosporidium* species (two mammalian, two avian, one reptilian, and one fish species) but indicates that additional studies are needed to confirm their validity. Fayer et al. (1997b) list eight valid named species of *Cryptosporidium*, listed in Table 2-1 (four mammalian, two avian, one reptilian, and one fish species), as well as referring to several unnamed species isolated from a variety of hosts.

C. parvum appears to be infectious to at least 79 species of mammals (O'Donoghue 1995) including humans (O'Donoghue 1995, Goodgame 1996, Fayer et al. 1997b). *C. muris*, the first mammalian species to be described (Tyzzer 1907), has so far not been identified in humans although it has been identified in a number of animals, including mice, rats, cats, dogs, cattle, and camels (Tzipori and Griffiths 1998). One report (the only one of its kind) found evidence that *C. baileyi*, which infects birds, was present in the stools and autopsied organs of an immunodeficient patient (Ditrich et al. 1991), but apparently the oocyst was actually *C. parvum* (Fayer 1998).

Tzipori and Griffiths (1998) review the difficulties associated with dividing *Cryptosporidium* into valid species: the lack of clearly defined and fully characterized reference strains for comparative studies to define distinguishing phenotypic and genotypic parameters; the ability of *Cryptosporidium* to infect a variety of cells, tissues, organs, and vertebrate species; and the often conflicting results of cross-transmission experiments. They suggest that *Cryptosporidium* should be seen as a genus consisting of a wide spectrum of isolates whose differences—of host origin, site of infection, and oocyst size—are not as important as virulence and genetic attributes, which have not yet been fully characterized.

Table 2-1. *Cryptosporidium* in Host Species

Valid named species of *Cryptosporidium*	
***Cryptosporidium* species**	**Host species**
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	*Homo sapiens* (humans)
	Mus musculus (house mouse)
C. serpentis	*Elaphe guttata* (cornsnake)
	Elaphe subocularis (rat snake)
	Sanzinia madagascarensus (Madagascar boa)
C. wrairi	*Cavia porcellus* (guinea pig)

Source: Adapted from Fayer et al. 1997b.

Tzipori and Griffiths (1998) performed pairwise comparisons on the few small subunit ribosomal RNA nucleotide sequences of *C. parvum, C. muris, C. baileyi,* and *C. wrairi* genes available in GenBank. The authors reasoned that the nucleotide sequences of isolates that have been identified as different species ought to be more different from one another than sequences of isolates of the same species. However, the analysis, which was based on only a few isolates, indicates that isolates that have been termed different species are not more different from each other than isolates within the same species (Tzipori and Griffiths 1998). Figure 2-1 illustrates the interspecies vs intraspecies relationship among isolates of *Cryptosporidium* as compared with three species of *Plasmodium*.

Considerable genotypic (genetically determined) and phenotypic (observable) differences exist among and within *C. parvum* isolates (Tzipori and Griffiths 1998). Variations in infectivity to other animals, pathogenicity, protein banding, antigenicity, isoenzyme typing, and genotyping have been recognized. Analysis of *C. parvum* genotypes using different genetic markers has shown that some genetic profiles from human and bovine isolates differ (Bonnin et al. 1996; Carraway et al. 1996, 1997). This observation could indicate that there are *C. parvum* isolates that are transmitted exclusively from human to human; for example, the heterogeneity of the *C. parvum* population infecting individual hosts would allow selection of different subpopulations, depending on the host species (Carraway et al. 1994, 1996). Alternatively, the difference in genetic profiles could imply that genetic markers in *C. parvum* can change upon passage to a different host.

The taxonomy of *Cryptosporidium* has been reviewed by Levine (1984, 1985), Current (1986), Fayer and Ungar (1986), Fayer et al. (1990), Fayer et al. (1997b), and Tzipori and Griffiths (1998).

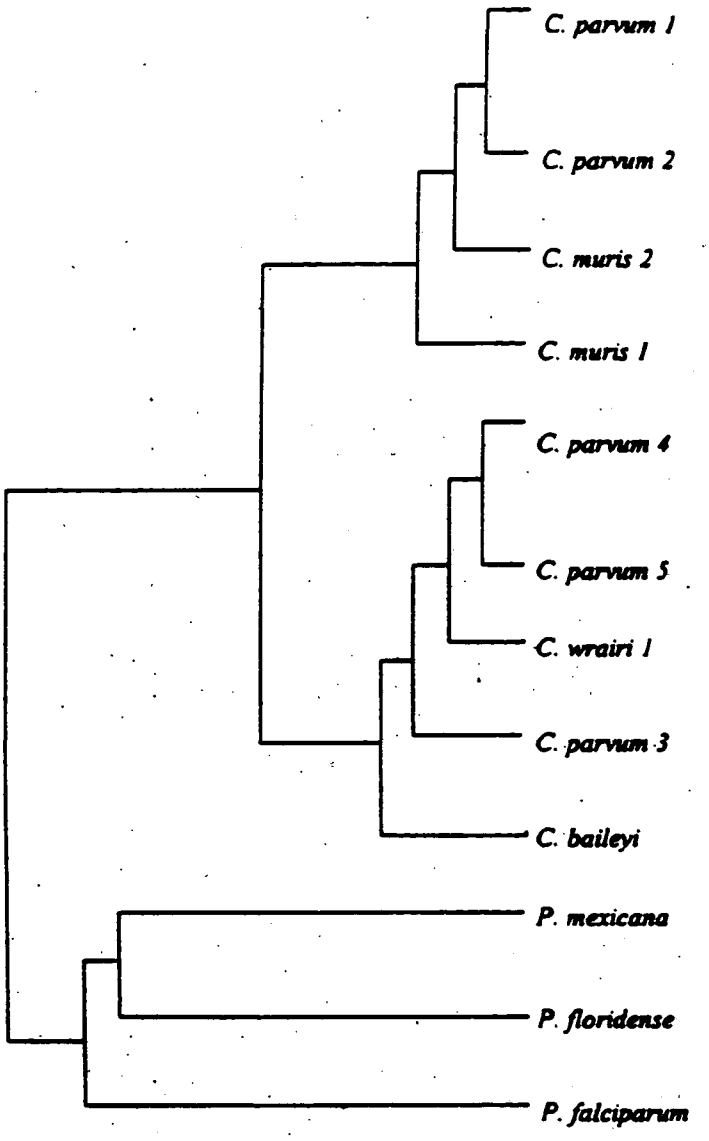


Figure 2-1. Dendogram Illustrating the Interspecies Versus Intraspecies Relationship.
Source: Tzipori and Griffiths 1998.

While *C. parvum* is a well-documented human pathogen, DuPont et al. (1995) noted that the virulence among isolates may vary. Chappell et al. (1997) have confirmed by laboratory analyses that there is a DNA fragment pattern difference between isolates. They have also demonstrated a significantly higher infectivity of one isolate (TAMU) as compared with another (Iowa) in cultured HCT-8 cells and in parallel human infectivity studies (Chappell et al. 1997).

Tzipori and Griffiths (1998) suggest that linking clearly recognized virulence attributes to stable genetic markers is a better method for defining a species. They conclude that until such definition is possible, "it will be wise to regard *Cryptosporidium* isolates from all vertebrates as a potential risk to public health."

2.1.2 Host Range

Cryptosporidium is ubiquitous and has been described (see Sect. 2.1.1) in a large number of host species (Fayer and Ungar 1986, Current 1986, Casemore 1990). Levine (1984) tentatively nominated *C. muris* as the isolate from mammals, *C. meleagridis* from birds, *C. crotali* from reptiles, and *C. nasorum* from fish. He assigned to the latter species all other animal isolates. Fayer et al. (1990) indicated that six species are valid representatives: *C. nasorum* in fish, *C. serpentis* in snakes, *C. meleagridis* and *C. baileyi* in birds, and *C. muris* and *C. parvum* in mammals. Fayer et al. (1990) further stated that *C. parvum* appears to be infective for all mammalian species including humans. Suitable hosts for *C. parvum* include humans; domesticated animals, mainly cattle, sheep, goats, swine, cats, and dogs; and wild animals, including deer, raccoons, foxes, coyotes, beavers, rabbits, and squirrels. Evidence of cross-species transmission is presented in Sect. 4.2.1.

2.1.3 Life Cycle

The life cycle of *Cryptosporidium* resembles that of other coccidia (Navin and Juranek 1984); it is monoxenous, meaning it completes its life cycle within one host without the need for a secondary host (Fayer and Ungar 1986, Current 1986, Casemore 1990, Butler and Mayfield 1996).

Oocysts, 4 to 6 µm in diameter, that are released into the environment via feces are very resistant to environmental stresses. Infected calves and lambs shed approximately 10 billion oocysts daily between 4 and 14 days post-infection (Blewett 1989b). Eighteen human volunteers were infected with challenge doses of *C. parvum*. The mean of total oocyst excretion during the course of the infection for subjects receiving a particular challenge dose ranged from 55,000 to approximately 800 million oocysts (Chappell et al. 1996).

Through fecal contamination of food or water, or by direct contact, oocysts are ingested by suitable hosts. In the gastrointestinal tract of such hosts, sporozoites excyst from the oocyst and parasitize epithelial cells. Thick-walled oocysts that are shed from the host in fecal material are resistant to environmental destruction and may persist until they enter a new host by the oral route. The excystation process releases four sporozoites, each of which may initiate a new life cycle by infecting the host. It has been shown that approximately 20 percent of the oocysts produced in the gut fail to form an oocyst wall and instead released "thin-walled oocysts." These thin-walled oocysts allow the excystation of sporozoites while still within the gut and result in accelerated infection of new cells [www.ksu.edu/parasitology/basicbio (Kansas State University, October 1997)].

The time period elapsed from ingestion to excretion of newly developed oocysts of *C. parvum* ranges from 2 to 7 days in calves, 2 to 14 days for dogs, 3 to 6 days for pigs, 2 to 5 days for lambs, and 4 to 22 days for humans. Also, the duration of shedding or oocyst excretion of *C. parvum* has been observed to be 1 to 12 days for calves, 3 to 33 days for dogs, 5 to 14 days for pigs, up to 56 days for primates, 1 to 20 days for immunocompetent humans, and up to six weeks for individuals with AIDS (Fayer 1997, Goodgame et al. 1993, Miller et al. 1990). In fact, Miller et al. (1990) observed partial acquired immunity in primates after reinoculation within two weeks following resolution of the primary infection. The primates did not exhibit any clinical symptoms of illness after reinoculation and shed very small numbers of oocysts intermittently compared with the initial primate shedding after the initial inoculation.

2.1.4 Fate and Transport in Environmental Media

The fate and transport of pathogens in the environment are major issues with respect to the exposure of humans to waterborne pathogens. The ability of microorganisms to survive in the environment allows them to be transported either by water, food, or personal contact to a human host (Hurst 1997). In this rule making, the human exposure pathway is via drinking water, both surface water and groundwater supplies. Surface waters that provide drinking water come from lakes, rivers, reservoirs, and cisterns. Groundwater supplies are extracted for drinking water by both vertical and horizontal wells, infiltration galleries, and springs.

2.1.4.1 Surface Water

Whenever the amount of rainfall is greater than can immediately be absorbed by the soil or soil cover, water will pond on the surface and, with increasing rainfall, run off to a lower point on the surface, such as a river, lake, or reservoir. Partly for this reason, cryptosporidiosis tends to be seasonal, with a higher prevalence during the warmest, wettest months (Current 1986). In most areas of North America, *Cryptosporidium* generally becomes a concern in surface waters between March and June, when spring rains increase runoff and many newborn animals are present in the environment to amplify oocyst numbers [www.ksu.edu/parasitology/basicbio (Kansas State University, October 1997)]. Soil particles can be suspended in this surface water and transported as surface runoff. The microorganisms (including parasitic protozoa) associated with the soil can be transported either as individual organisms, aggregates of organisms, or within an aggregate of soil particles and organisms.

Little published information exists about the significance of extreme events, runoff, and sediment resuspension on source water densities of *Cryptosporidium*. Recently, LeChevallier et al. (1997a) and Stewart et al. (1997a) studied the effects of runoff events on *Cryptosporidium* and *Giardia* densities and found the greatest protozoan densities were detected during the "first flush" following a rainfall or source water turbidity spikes.

The character (topography, plant cover) and uses (urban, farming) of a watershed also influence the occurrence or concentration of *Cryptosporidium* in surface water (Hansen and Ongerth 1991). For example, a mountainous forested watershed with little or no human activity had the lowest surface water oocyst concentrations and oocyst production, while downstream sample sites influenced by dairy farming and urban

runoff had oocyst concentrations and production rates almost 10 times higher than the upstream sites (Hansen and Ongerth 1991).

Cryptosporidium can be transported not only in surface runoff, but also through soil and groundwater or land drains to surface water (Mawdsley et al. 1996, Hurst 1997). Movement of *Cryptosporidium parvum* through soil and groundwater is influenced by filtration, adsorption to soil and aquifer matrix particles by processes that are governed primarily by the magnitude and distribution of the electrical charge on the organism and the surrounding soil and aquifer matrix, and sedimentation within soil or aquifer pores.

The tendency of microorganisms to adsorb onto suspended particles facilitates the sedimentation of those organisms during periods of low water flow or low runoff. Those sediments then serve as reservoirs from which the organisms can be resuspended during periods of intense rainfall and/or flooding (Wilkinson et al. 1995). For example, oocysts in feces deposited on soil surfaces are readily transported during rainfall by surface runoff into surface water. In addition, during periods of increased source water turbidity and elevated flow, source water densities of *Cryptosporidium* were elevated compared with normal conditions (LeChevallier et al. 1997a). Whether this was due to runoff or resuspension of sediment from scouring effects was not determined, but the results suggest the potential for contribution of *Cryptosporidium* oocysts by sediment resuspension.

Factors that limit an understanding of environmental fate and transport of *Cryptosporidium* include a lack of information on *in situ*, micro-scale environmental factors, and the fundamental biological properties of oocysts that affect their transport and survival in watershed environments (Anguish and Ghiorse 1997). Another factor that limits understanding about the fate and transport of these organisms is the apparent buoyancy of oocysts. Although in source waters they may be bound to particulate matter, oocysts that are unbound may have a tendency to float. Researchers have found that 65 - 83.9 percent of oocysts floated up into the supernatant of a homogenized fecal matter/distilled water mixture after 18 and 23 hours (Swabby-Cahill et al. 1996). Also, it was discovered that after 6 spins of 10 minutes at 1500 rpms, a total of 16 percent of the oocysts were detected in the top third of the supernatant suggesting some resistance to settling (Swabby-Cahill et al. 1996). *Cryptosporidium* oocysts have been shown to have a very low density (about 1.05 g/cm^3) and a very low settling rate (2 mm per hour or less), which suggests that sedimentation may not be a significant source of oocyst removal (Gregory 1994). Rose et al. (1997a) also noted the low sedimentation rate for oocysts.

Although overland transport of oocysts in feces and manure from domestic farm animals and infected wildlife into surface water has been suggested as a source of oocysts in rural watershed areas (Madore et al. 1987, Ongerth and Stibbs 1987, Rose 1988), the actual mechanisms of oocyst survival and transport in the environment are poorly understood (Anguish and Ghiorse 1997). An improved understanding of transport mechanisms is further hindered by the lack of an effective method to detect oocysts in various silt- or clay-laden water matrices.

2.1.4.2 Groundwater Under the Direct Influence (GWUDI) of Surface Water

Some groundwaters, extracted for drinking water supply by wells, infiltration galleries, or springs, are regulated as surface water. These groundwater supplies are considered to be especially vulnerable to contamination by parasitic protozoa. Groundwater that is considered to be groundwater under the direct influence of surface water (GWUDI) is usually immediately adjacent to surface water or to the discharge point of a spring. GWUDI may be contaminated by direct infiltration of oocysts from the surface as a result of transport by infiltrating precipitation. More likely, however, groundwater may be contaminated by the action of pumping wells. Given sufficiently high pumping rates, wells can reverse the normal groundwater flow direction. In this case, surface water is induced to flow from a river, lake, or reservoir into the adjacent groundwater, where it may be extracted by one or more pumping wells. If the surface water is contaminated by oocysts, then the adjacent groundwater may also contain oocysts and, therefore, is regulated as if it were surface water.

To understand the likelihood that oocysts could be transported through soil to groundwater, Mawdsley et al. (1996) studied transport of *Cryptosporidium parvum* oocysts through three soil types. Oocysts were detected in leachate (water collected from the bottom of the soil column) from intact soil columns following a 21-day irrigation period. Although most (72.8 percent) of the oocysts were found in the top 2 cm of soil, 5.36 percent were found at soil depths of 30 cm. *C. parvum* transport through soil was greater in a silty loam and a clay loam soil than in a loamy sand soil. Evidence suggests that the extent of adsorption is greater and the size of micropores is smaller in clay than in sandy soils, so the results suggest that factors other than adsorption and micropore size influenced the oocyst movement. The authors' use of intact soil cores maintained, it is believed, the natural soil structure and macropores. They conclude that the rapid flow of water through macropores, which are representative of natural field conditions, largely bypasses the filtering and adsorptive effects of the soil, greatly increasing the risk of pathogen transport to groundwater (Mawdsley et al. 1996).

The induced infiltration of *Cryptosporidium* oocysts by pumping wells has not been directly studied, although the occurrence of oocysts in well water suggests that such transport has occurred. EPA (1997b) identified *Cryptosporidium* (some are presumptive positive samples rather than confirmed oocysts) in 17 vertical wells and 5 horizontal wells. [Most, if not all, of these wells are also reported in Hancock et al. (1998b)].

It is believed that the surface water sediments and the aquifer matrix material play a significant role in minimizing oocyst transport to the wells. However, there is insufficient information about the hydrogeology of the aquifer matrix and sediments to determine the nature of those media and their significance in preventing contamination. Because of the difficulty in recovering oocysts from water samples, it is not known whether the oocysts are present and not recovered or are simply not present in common hydrogeologic settings, such as alluvial aquifers. In addition, as discussed in Sect. 3.1.2, problems with detection methods complicate data collection. Furthermore, little information is available to elucidate which hydrogeologic settings are sensitive to oocyst contamination because groundwater flow and oocyst transport through fractures or dissolution conduits effectively bypass the protective action of most of the aquifer matrix.

Hancock et al. (1998a) investigated the correlation of *Cryptosporidium* and *Giardia* occurrence in groundwater with microbial surface water quality indicators. The presence of *Giardia* correlated with the presence of *Cryptosporidium*. Occurrence of *Giardia* was correlated with source depth, but there was no correlation between *Cryptosporidium* occurrence and source depth. There was a correlation between general risk categories of low, moderate, and high and the occurrence of *Cryptosporidium* and *Giardia*, but specific data have not yet been published. However, there was no correlation between the distance of the groundwater source from adjacent surface water and either pathogen (Hancock et al. 1998a).

Gollnitz et al. (1997) studied the algae reduction efficiency of a porous-media aquifer in Casper, Wyoming. The public utility withdraws groundwater from vertical wells, horizontal collectors wells, and an infiltration gallery in an alluvial wellfield. Results from multiple samplings over more than three years demonstrated greater than 4-log reduction of algae through the aquifer; no *Giardia* or *Cryptosporidium* was recovered from any of the collection devices during the sampling period.

Harvey et al. (1995) modeled the transport of protozoa in groundwater systems, using free-living flagellates (2 to 3 μm *in situ*) and microspheres 0.7 to 6.2 μm in size. They noted that physical straining was not of major importance in porous media, such as coarse sands, with grain diameter greater than 100 μm. Adsorption or adherence to surfaces appeared to be reversible. The largest microspheres (2.8 and 6.2 μm) were not significantly transported, but 1.7-μm-size microspheres were present in the effluent from the column study at 83 percent of the level of the injected concentration level. Rose (1997) noted that, according to a personal communication from C.P. Gerba, *Cryptosporidium* oocysts measuring 5 μm behave in filtration studies more like a 2 μm-sized microsphere, possibly owing to biological flexibility.

Additional studies are in progress to understand the factors contributing to *Cryptosporidium* transport in the subsurface. Wagner et al. (1997) are attempting to characterize the transport behavior of *C. parvum* in saturated artificial and field sediments. Their experiments are designed to determine the filtration coefficient of *C. parvum* and its dependency on physical properties of the porous medium such as mean grain size and fluid velocity. Results may determine whether colloid filtration theory can be used to simulate *C. parvum* transport in saturated soils and groundwater (Wagner et al. 1997). Darnault et al. (1997) are studying preferential flow paths of *C. parvum* through several different media: glass bead columns, sand columns, and disturbed and undisturbed soil columns. Results indicate that *C. parvum* can flow through the vadose zone (Darnault et al. 1997).

2.1.5 Environmental Persistence and Viability of Oocysts

A variety of factors influence the survival in the environment of oocysts and other enteric pathogens. Marginally treated or untreated surface water supplies result in high risk of transmitting waterborne disease (Craun 1990) because oocysts can survive for several months in cold waters (Robertson et al. 1992), and relatively low numbers of organisms are required for an infective dose (DuPont et al. 1995).

Heisz (1997) tested the effects of water chemistry and season on the survival of *Cryptosporidium* oocysts in water from three different rivers in Canada. Purified oocysts were inoculated into test waters and synthetic hard water (100 ppm as $CaCO_3$, pH 7.0) in microtubes at an initial concentration of 5×10^5

oocysts/mL and incubated at 20°C and 30°C for water samples obtained during the summer season (July-September, 1995) and 4°C and 20°C for samples obtained during the winter season (November-December, 1995). All samples were kept in the dark, and viability was determined by *in vitro* excystation assay. Inactivation rates were significantly correlated with incubation temperature and heterotrophic plate count. Analysis of summer sample inactivation rates showed that after 50 days incubation, a portion of the oocysts was still viable. There was, at most, a 2.5-$\log_{10}$ reduction in oocyst viability over 30 days incubation. Winter samples incubated at 4°C and 20°C showed at most a 1.2-$\log_{10}$ reduction in viability over a 30-day incubation period. All natural waters had lower oocyst survival/viability rates than the oocysts incubated at the same temperatures in synthetic hard water. Samples incubated at 4°C showed similar viability rates compared with synthetic hard water controls, while samples at 20°C showed slightly decreased rates compared with controls. The data indicated that microbiological activity within natural waters may have reduced *Cryptosporidium* oocyst survival, especially at higher water temperatures of 20°C and 30°C when there is increased biological activity within the water compared with activity at 4°C (Heisz 1997).

Johnson et al. (1997) studied the effect of light on oocyst survival in marine waters, using excystation as the viability assay for *C. parvum*. Robertson et al. (1992) has shown that *Cryptosporidium* remains viable for greater than 35 days at 4°C in the dark in seawater. Johnson et al. (1997) found that oocysts were inactivated by 1 log by day 2 when exposed to sunlight but, in the dark, greater than 97 hours were required for the same 1-log reduction in marine water.

Microorganisms are inactivated in soil at rates that vary with the degree of predation by other microorganisms, the amount of sunlight, and the physical and chemical composition of the soil, including moisture content, pH, and temperature (Gerba et al. 1975, Kowal 1985). However, few studies have examined the fate of oocysts in the environment (Rose 1997), especially survival in saturated and unsaturated soils and in groundwater. Because animals may excrete large numbers of oocysts in a day (Rose 1997), there is a real potential for contamination of groundwater in hydrogeologic settings where the normal protective action of the soil and aquifer matrix is bypassed by flow through macropores, fractures, or conduits. Representative examples of effects of environmental variables on oocyst viability are summarized in Table 2-2.

2.2 *GIARDIA* AND *GIARDIA LAMBLIA*

Giardia is a parasite with worldwide distribution, and giardiasis is one of the most prevalent intestinal diseases of humans (Meyer 1990, Craun 1986). In addition, infection with *Giardia* is common among domestic animals, such as cats, dogs, birds, horses, rabbits, sheep, cattle, and goats, as well as other mammals and birds (Meloni et al. 1995). Evidence for zoonotic transmission (zoonosis: a disease acquired by humans from animals) was developed by Meloni et al. (1995) in their study of genetic variation of *Giardia*.

2.2.1 Taxonomy

Members of the genus *Giardia* are flagellated, single-celled, binucleate protozoans that parasitize the intestinal tract of virtually every class of vertebrates. These one-celled protozoans have a two-stage life cycle,

Table 2-2. Survivability of *Cryptosporidium* Oocysts in the Environment

Environmental condition	Experimental notes	Effect	Viability assay	Reference
Cleaned oocysts in distilled water	72.4°C for 1 min; 64.2°C for 2 min	Not infective	Mouse infectivity	Fayer 1994
Cleaned oocysts in distilled water	-15°C for 7 days; -20°C for 8 hours; -70°C for 1 hour	Not infective	Mouse infectivity	Fayer and Nerad 1996
Cleaned oocysts in distilled water	5°C for 7 days; -10°C for 7 days; -15°C for 24 hours	Infective	Mouse infectivity	Fayer and Nerad 1996
Oocysts	Moist heat at 45°C for 20 min; 60°C for 6 min	Not infective		Anderson 1985
Calf feces	Air drying, 1–4 days	Not infective	Mouse infectivity	Anderson 1986
Oocysts in reverse osmosis water	Snap-frozen in liquid nitrogen	100% dead	Excystation; DAPI/PI	Robertson et al. 1992
	Slow frozen at -22°C for 21 hours	67% dead	Excystation; DAPI/PI	Robertson et al. 1992
	Air drying at room temperature for 2 hours	97% dead	Excystation; DAPI/PI	Robertson et al. 1992
	Air drying at room temperature for 4 hours	100% dead	Excystation; DAPI/PI	Robertson et al. 1992
Human stools	4°C for 178 days	41–>99% dead	Excystation; DAPI/PI	Robertson et al. 1992
Cow feces	5–10°C for 176 days	60–72% dead	Excystation; DAPI/PI	Robertson et al. 1992
Tap water	5–10°C for 176 days	96–99% dead	Excystation; DAPI/PI	Robertson et al. 1992
River water	5–10°C for 176 days	89–99% dead	Excystation; DAPI/PI	Robertson et al. 1992
River water	Summer samples, 50 days	Maximum 2.5-log reduction in viability	Excystation	Heisz 1997
	Winter samples, 4°C and 20°C, 30 days	Maximum 1.2-log reduction in viability	Excystation	Heisz 1997

Table 2-2 (continued)

Environmental condition	Experimental notes	Effect	Viability assay	Reference
Seawater	4°C for 35 days, in the dark	33-44% dead	Excystation; DAPI/PI	Robertson et al. 1992
Marine water	22–27°C for >97 hours, in the dark	90% inactivated	Excystation	Johnson et al. 1997
	22–27°C for 48 hours, in the sunlight	90% inactivated	Excystation	Johnson et al. 1997

DAPI/PI = diamidino-2-phenylindole/propidium iodine (dye exclusion).

the trophozoite (vegetative form) and the cyst (dormant, resistant form). Trophozoites of *Giardia lamblia* (synonyms: *G. duodenalis* and *G. intestinalis*) inhabit the upper small intestine of the vertebrate host (Filice 1952). The trophozoites and cysts measure 9 to 21 μm and 8 to 12 μm in length, respectively (Daly 1983, CDC 1995b, EPA 1996a).

Either host specificity or morphological characteristics have been used to distinguish species of *Giardia* (Meloni et al. 1995). However, both approaches have limitations. Additional research is ongoing to try to provide a taxonomic interpretation of genetic variation. For example, Meloni et al. (1995) used enzyme electrophoresis to reveal extensive genetic variation of isolates of *Giardia duodenalis*. Results showed genetically identical isolates from humans and other animals and extensive genetic diversity among isolates from humans (Meloni et al. 1995). Thompson and Lymbery (1996) examined genetic variability, in *Giardia* and *Cryptosporidium*, and noted the lack of understanding about within-host interactions between genetically different parasites, both within the same species of parasite and among different species of the same genus. Hopkins et al. (1997) compared small subunit ribosomal RNA sequences from 13 human and 9 dog isolates of *Giardia duodenalis* and revealed 4 different genetic groups. Groups 1 and 2 contained all of the human isolates, whereas groups 3 and 4 consisted entirely of *Giardia* samples recovered from dogs. A genetic basis for the differences observed between the groups was supported by sequence analysis of 9 *in vitro* cultured isolates that were placed into the same genetic groups established by enzyme electrophoresis (Hopkins et al. 1997).

2.2.2 Host Range

Giardia can infect a wide variety of wild and domestic animals (Craun 1990). Experimental animal infection studies have shown that many *Giardia* are not highly host specific. *Giardia* infections can be transmitted from humans to other animals as well as from other animals to humans. Meloni et al. (1995), Thompson and Lymbery (1996), and Hopkins et al. (1997) have used molecular techniques to attempt to elucidate complex host-parasite interactions and zoonotic relationships. A discussion of cross-species transmission is included in Sect. 4.2.1.

2.2.3 Life Cycle

The life cycle of *Giardia* is considered to be simple and direct because no intermediate hosts are required. Trophozoites attach to the brush border of the small intestine by their ventral adhesive disks. Reproduction of trophozoites is by binary fission. The transformation from trophozoite to cyst begins in the small intestine when the trophozoite detaches from the epithelium, becomes round in shape, and develops a cyst wall. The resultant cyst is excreted with the feces. *Giardia* survive passage outside the host as cysts, and a new host acquires the infection by ingestion of the cysts (EPA 1996a).

2.2.4 Fate and Transport in Environmental Media

The factors that influence the transport of *Giardia* in the environment are the same as those affecting *Cryptosporidium* (see Section 2.1.4): adsorption, filtration, and sedimentation. The other main feature affecting transport of *Giardia*, especially in soil and aquifer materials, is its size. The *Giardia* cyst size is 8 to 12 μm in diameter, larger than the 4- to 6-μm *Cryptosporidium* oocyst. The larger size of the cyst potentially restricts movement through some soils and aquifer materials, except in the presence of natural pathways such as macropores, fractures, and conduits. As with *Cryptosporidium*, *Giardia* cysts in feces deposited on soil surfaces are readily transported during rainfall by surface runoff into surface water and, perhaps, in some hydrogeologic settings, to groundwater.

2.2.4.1 Surface Water

As detailed in this report, *Giardia* cysts are ubiquitous in the environment, have a number of mammalian reservoir hosts in addition to humans, and are resistant to environmental exposures. Their ability to survive (Section 5.3) in surface water, even pristine water samples (Roach et al. 1993), allows the cysts to be transported significant distances in flowing water to other potential hosts (Hurst 1997). The size difference between *Giardia* and *Cryptosporidium* is likely not significant in governing the transport of cysts, as opposed to oocysts, in surface runoff. It is not clear if there are significant differences in the surface charge of the two organisms and, if so, whether the transport of cysts versus oocysts in surface runoff would differ. In many cases, the discussion about factors affecting fate and transport of *Cryptosporidium* in surface water (Section 2.1.4.1) is also applicable to *Giardia* transport (Hansen and Ongerth 1991; Crockett and Haas 1995a,b, 1997).

2.2.4.2 Groundwater Under the Direct Influence of Surface Water

With the exception of the Hancock et al. (1998a) investigation discussed in Sect. 2.1.4.2, no information is available to contrast the differing transport of *Giardia* cysts versus *Cryptosporidium* oocysts through soils or sediments to groundwater, with or without the presence of macropores, fractures, or conduits. It is likely that the difference in size and any possible difference in charge between the two could affect the relative rate of transport of each through soils, sediments, and aquifer materials. Mikels (1992) found that no *Giardia* were recovered from horizontal collector wells constructed in alluvial river valleys, although *Giardia* were detected in adjacent rivers. In addition, no insects, other macroorganisms, or other large-diameter pathogens were present in the water from those collector wells.

2.2.5 Environmental Persistence and Viability of Cysts

Environmental conditions contributing to the persistence of *Giardia* cysts are similar to those described for *Cryptosporidium* in Section 2.1.5. Surface water sources are more likely to be contaminated with *Giardia* than are groundwater sources (Craun 1990). Marginally treated or untreated surface water supplies result in high risk of transmitting *Giardia* because cysts can survive for several months in cold waters, and relatively low numbers of *Giardia* are required for an infective dose (Craun 1990). Table 2-3 summarizes some representative examples of the effects of environmental conditions on the persistence and viability of *Giardia* cysts.

Table 2-3. Effects of Environmental Conditions on the Viability of *Giardia* Cysts

Environmental conditions	Experimental notes	Effect	Viability assay	Reference
Liquefied feces	Stored at 4°C	Infective for 1 yr	Infectivity in rats determined by microscopy of feces, counterimmuno-electrophoresis of feces for antigen, and intestinal biopsy	Craft 1982
Distilled water	Stored at 37°C	Viable ≤4 days	Excystation *in vitro*, and excystation vs eosin exclusion	Bingham et al. 1979
Distilled water	Stored at 8°C	Viable 77 days	Same as above	Bingham et al. 1979
Tap water	Cysts in tap water subjected to 100°C	Immediately incapable of excystation	Same as above	Bingham et al. 1979
Tap water	Cysts in tap water at -13°C	<1% survived freezing for 14 days	Same as above	Bingham et al. 1979
Distilled water	*G. muris* cysts in fecal pellets stored in water in refrigerator at 5–7°C; viability tested by mouse infectivity	100% viability at 7 days; 17–100% at 28 days; 0% at 56 days	Fluorogenic dye exclusion, production of giardiasis in an animal, and cyst morphology by microscopy	deRegnier et al. 1989
Environmental waters (lake and river)	*G. muris* cysts in fecal pellets stored in water in refrigerator at 5–7°C	Viable 2–3 months	Same as above	deRegnier et al. 1989

Table 2-3 (continued)

Environmental conditions	Experimental notes	Effect	Viability assay	Reference
Minneapolis tap water	*G. muris* cysts in fecal pellets stored in glass vials suspended in flowing tap water at 20–28°C	Loss of viability within 3 days; 0–17% viable at 7 days based on mouse infectivity; no viable cysts at 14 days	Same as above	deRegnier et al. 1989
Artificial sea water	24 hr at 4°C	No cysts survived	Excystation *in vitro*	Jarroll et al. 1984
Marine waters	3 hr in marine waters in sunlight	3-log reduction in viability	Excystation	Johnson et al. 1997
	77 hr in marine waters in the dark	3-log reduction in viability		Johnson et al. 1997
Air	Cysts exposed to air drying at 4°C or 21°C for 24 hr	No cysts survived	Same as above	Jarroll et al. 1984

The persistence of *Giardia* cysts in water is well documented (Madore et al. 1987, Craun 1990, Hancock et al. 1998b, LeChevallier and Norton 1995). *Giardia* cysts survive relatively long periods in water, particularly at temperatures below 20°C; above 20°C, cyst inactivation is rather rapid (Jakubowski 1990). Evidence suggests that *Giardia* cysts in fresh water survive best at 4 to 8°C (Jakubowski 1990). Kayed and Rose (1987) reported survival of protozoan cysts in water in the laboratory for greater than 140 days. Jarroll et al. (1984) reported that cysts did not survive when *Giardia* were exposed for 24 hours to artificial sea water at 4°C or to air-drying at 4°C or 21°C. Johnson et al. (1997) studied the survival of *Giardia*, *Cryptosporidium*, and other enteric pathogens in marine waters. Using excystation as the viability assay, they demonstrated a 3-log reduction of cysts in marine waters after 3 hours in direct sunlight; but cysts in the dark required 77 hours to show a 3-log reduction (Johnson et al. 1997).

3. DETECTION AND ENUMERATION OF PATHOGENS

The ICR (61 *FR* 24353) requires that public water systems supplying surface water to at least 100,000 people must monitor raw water for disease-causing organisms including *Giardia*, *Cryptosporidium*, and viruses. If *Giardia* or *Cryptosporidium* is detected at greater than 10 organisms/L or total culturable virus is detected at greater than 1/L in source water, treated water must also be monitored. Monitoring must continue for a total of 18 months. The ICR method requires the use of an indirect fluorescent antibody (IFA) assay for the identification and enumeration of *Giardia* and *Cryptosporidium* (EPA 1996b). In accordance with requirements of the ICR, the equivalent volume of water analyzed (volume of water filtered times fraction of filter pellet examined) must be at least 10 L.

To meet EPA's goals for rates of infection, the SWTR (54 *FR* 27486) and the proposed IESWTR (59 *FR* 38832) target concentrations of viable *Giardia* cysts around 0.01 to 0.001 per 100 L in treated drinking water. Similar goals for *Cryptosporidium* oocysts were also discussed in the proposed IESWTR. EPA also proposed alternative treated water goals of higher concentrations (59 *FR* 38832), depending upon risks that might be associated with protozoan concentration levels and what EPA determined to be appropriate as an acceptable level of risk. Currently available methods for parasites and viruses are not able to reliably measure such treated water concentrations. The following sections describe the analytical method for these pathogens that has been approved for the ICR and discuss its advantages and limitations in meeting the objective of sensitive and reliable real-time monitoring.

3.1 DIRECT MEASUREMENT METHODS

Protozoa and viruses typically occur at low concentrations in source water and in treated drinking water, making it usually necessary to concentrate the sample to make detection reliable. Filtration is most often used to concentrate the organisms. Methods for analysis of the concentrated suspensions are specific for the type of pathogen. For example, direct methods for the detection of protozoan pathogens require identification of the organisms by microscopic examination. In contrast, pathogenic viruses are often identified by their characteristic cytopathic effect (CPE) on cell cultures.

The ICR method that is described below will be used to generate the data from which the national occurrence estimate will be derived. It was developed from D-19 Proposal P229 (ASTM 1992) and proposed Method 9711B (Eaton et al. 1995). Other methods are described in Appendix A.

3.1.1 Description of ICR Standard Method

Most methods for the analysis of water for *Cryptosporidium* have been based on methods for *Giardia*. However, recovery of *Cryptosporidium* is more difficult because *Cryptosporidium* oocysts (4 to 6 μm) are smaller than *Giardia* cysts (8 to 12 μm) and also tend to stick to the filter fibers. Cysts and oocysts are concentrated by filtration, eluted from the filter, recovered from the eluate by centrifugation, and separated from some of the particulate debris by differential centrifugation in a density gradient. The partially purified

cysts and oocysts are deposited on a cellulose-acetate membrane and stained to enable microscopic identification and enumeration.

The following sections describe the ICR method. This description is given in some detail because it is the standard method for the ICR. Less detailed descriptions of some of the precursors of the ICR method, as well as of other methods that have been used or are under development, are presented in Appendix A.

3.1.1.1 Summary

The ICR method provides a quantitative measurement of contamination of water with *Giardia* cysts and *Cryptosporidium* oocysts. The method does not identify species of the protozoa, nor does it determine viability or infectivity of the organisms that are detected. In the ICR method (EPA 1996b), a large volume of water, usually 100 L (26.4 gal) of source water or 1,000 L (264 gal) of finished water, is pumped through a polypropylene yarn-wound filter or Filterite cartridge with a nominal porosity filter size of 1 μm. Cysts and oocysts are removed from the filter fibers by washing them in an eluting solution; then they are centrifuged to pellet the cysts and oocysts. The pelleted material is then clarified, that is, separated from other debris by Percoll-sucrose density gradient centrifugation.

The partially purified cysts and oocysts are applied to a cellulose acetate membrane filter. The concentration of particles in the suspension is adjusted so that there is no more than a monolayer of particles on the filters (otherwise cysts and oocysts may be obscured by debris). Each filter is stained with a fluorescein-tagged antibody preparation that ideally reacts specifically with *Giardia* and *Cryptosporidium* and is then mounted on microscope slides in a mounting medium that makes the filter transparent.

Slides are examined under ultraviolet light to detect particles that fluoresce with an apple-green color and are round or ovoid, 4 to 6 μm (*Cryptosporidium*) or 8 to 12 μm (*Giardia*) in length. Each fluorescent body is then examined at 1,000× magnification, using Hoffman modulation® or differential interference contrast (DIC) optics, to confirm the absence of atypical structures (stalks, spikes, appendages, large nuclei, chloroplasts, crystals, spores, etc.). Fluorescing particles of the appropriate size and shape, and those lacking atypical characteristics, are classified and recorded as either empty *Giardia* cysts, *Giardia* cysts with amorphous internal structure, *Giardia* cysts with one or more internal structure, empty *Cryptosporidium* oocysts, *Cryptosporidium* oocysts with amorphous internal structure, or *Cryptosporidium* oocysts with internal structure (i.e., one to four sporozoites).

Concentrations of *Giardia* cysts and *Cryptosporidium* oocysts, expressed as numbers/100 L, are calculated using the number of cysts and oocysts identified, the fraction of the sample that was examined microscopically, and the volume of water sampled.

3.1.1.2 Advantages

One major advantage of the ICR method is that the method is described in great detail so that trained analysts should be able to achieve consistent processing techniques. Also, the method provides identification,

as well as enumeration, of *Giardia* cysts and *Cryptosporidium* oocysts; and the limit of detection depends only on the equivalent volume of water analyzed. The limit of detection is one *Giardia* cyst or one *Cryptosporidium* oocyst in the total volume tested. Increasing the volume tested lowers the limit of detection, increasing the likelihood of detecting pathogens that are present at low concentrations levels. The Method Detection Limit, a concentration level that is detectable with high probability, has not been determined for the ICR method, but is expected to be much greater than the limit of detection.

3.1.1.3 Limitations

Limitations to the effectiveness of the ICR method occur at every step of the method. Loss of sample can occur during filtration, elution of cysts and oocysts, concentration of the eluate, and clarification of the concentrate. Interferences can occur during staining and observation of the mounted specimens. Optimal recovery of cysts and oocysts requires that the technician is well trained and follows the method carefully. Identification of the immunofluorescent cysts and oocysts requires a skilled microscopist and expensive equipment. Each assay requires several hours of sample preparation, and results have shown to be highly variable (Clancy et al. 1994, Straub et al. 1997). The assay is sufficiently complex that it is unlikely to be performed in the laboratory of a small- or medium-sized water purification plant. Therefore, there are also delays resulting from sample transportation, data review, and reporting.

3.1.2 Uncertainties of Measurements

3.1.2.1 Recovery

The ICR method for *Giardia* and *Cryptosporidium* suffers from low and variable recovery. Analysis of performance evaluation (PE) samples illustrates the difficulty of the technique. A survey of 12 commercial laboratories showed an average of 9 percent recovery of *Giardia* cysts and 3 percent recovery of *Cryptosporidium* oocysts from spiked samples containing approximately 387 cysts and 326 oocysts per filter (Clancy et al. 1994). *Giardia* cysts were not detected by 4 of 11 laboratories surveyed, and 5 of 11 failed to detect *Cryptosporidium* (Clancy et al. 1994).

An evaluation of the ICR method by four laboratories showed mean recoveries of 36.7 and 26.4 percent for *Giardia* in raw and finished water, respectively, with a range of 1.8 to 124.6 percent. Mean recoveries of *Cryptosporidium* were 11 and 7.2 percent in raw and finished water, respectively, with a range of 0.3 to 53.0 percent (Hargy et al. 1996). Two subsequent PE studies showed mean recoveries of 25 and 44.4 percent for *Giardia* and 22.9 and 34.9 percent for *Cryptosporidium*, with recoveries ranging from 0 to approximately 140 percent for both organisms (Crockett and Haas 1997). Other data have shown a mean recovery rate of 42.4 percent for *Giardia* and 23.6 percent for *Cryptosporidium* from spiked samples (LeChevallier and Norton 1995), and mean oocyst recoveries of 21.5 percent from raw water and 14 percent from finished water (Hancock et al. 1998b, Klonicki et al. 1997). Even in a single laboratory, variability is high. For example, an evaluation of recovery of *Cryptosporidium* oocysts from spiked filters showed a mean of 24.2 percent, with a range of 15 to 35 percent and a standard deviation of 7.9 percent (LeChevallier et al. 1991c). Another study (Crockett and Haas 1997) showed a mean recovery of 17 percent for *Giardia* (range

16 to 19 percent) and 13 percent for *Cryptosporidium* (range 2 to 48 percent). It is possible that recovery depends, in ways that are not yet clear, on the amount of turbidity in the sample. For example, studies have shown recoveries to be 21 percent for raw water and 14 percent for finished water for *Giardia* (Hancock et al. 1998b, Klonicki et al. 1997), and recovery of *Cryptosporidium* oocysts from a variety of filters was generally lower when they were spiked into river water than when they were spiked into tap water (Shepherd and Wyn-Jones 1995, Whitmore and Carrington 1993). However, recovery of both *Giardia* and *Cryptosporidium* was higher in raw water with high turbidity (60 NTU) and total solids (320-1493 mg/L) than in raw water with low turbidity (1.9 NTU) and total solids (100-120 mg/L) (Hargy et al. 1996). The wide range of recoveries within a single laboratory (LeChevallier et al. 1995a) and among qualified laboratories (Clancy et al. 1994) indicated that initially not all properties of the methods, of the organisms and of different water matrices were fully understood.

Losses of cysts and oocysts occur during initial filtration of the sample. Organisms may pass through the filter (LeChevallier et al. 1991a) or may be incompletely eluted from the filter matrix (LeChevallier et al. 1991a). Losses during filtration are variable and seem to be greater with raw water than with finished water (Shepherd and Wyn-Jones 1995, Whitmore and Carrington 1993). Clarification steps introduce further losses of cysts and oocysts (Hancock et al. 1998b, Klonicki et al. 1997). The specific gravity of the Percoll-sucrose gradient appears to be particularly important (LeChevallier et al. 1991a).

Slide preparation and microscopic analysis also exhibit variability of recovery and of detection of cysts and oocysts. Straub et al. (1997) and Jackson et al. (1997) reported studies in which analysts applied known numbers of cysts and oocysts to cellulose acetate filters and carried out IFA staining and microscopic analysis of the filters. The average recoveries of *Giardia* cysts and *Cryptosporidium* oocysts were 69 percent (standard deviation ±20 percent) and 42 percent (standard deviation ±22 percent), respectively (Straub et al. 1997). In a similar study, Klonicki et al. (1997) found a 67.6 percent relative difference between cellulose acetate filter counts and hemacytometer counts of the same suspension. Some cysts and oocysts passed through or around the membranes (Straub et al. 1997). Shepherd and Wyn-Jones (1995) showed that recovery of oocysts was higher when concentrated oocysts were stained before being placed on the membranes, presumably because antibody-binding sites are more available when the oocysts are in suspension. However, Straub et al. (1997) showed that even when oocysts were stained in suspension and counted on well slides (with an average recovery of 97 percent), variability was high (relative standard deviation 21 percent).

EPA recognizes that the method is difficult to run, has poor recovery efficiencies, and does not have a high level of precision. Due to the limitations of the ICR method, EPA has restricted analysis of samples using this method to laboratories that meet stringent approval criteria. EPA will also limit the use of the ICR data to developing a national occurrence database and national cost impacts of regulatory options for the final ESWTR.

3.1.2.2 Method Detection Limit

The method detection limit of the ICR method has not formally been determined. The method detection limit is intended to be a value which is "the minimum concentration of a substance that can be

measured and reported with 99 percent confidence that the analyte concentration is greater than zero" (40 CFR 136, Appendix B). However, the statistical treatment of 40 CFR 136, Appendix B applies to chemical measurement methods and caution should be used when applying it to methods which employ counting of organisms or other particles in suspension. The variability of recovery of the ICR method further complicates the determination of a statistical method detection limit. Assuming that a sample does not become contaminated during processing and that the analyst correctly evaluates the sample under the microscope, the presence of one cyst or oocyst indicates the presence of parasites in the water from which the sample was taken. However, a negative result (no cysts or oocysts detected) is not conclusive evidence that cysts or oocysts are absent from the sample. The negative result may be a false negative, due to low recovery, variable recovery, small fraction of pellet examined, small volume of water filtered, or chance. The likelihood of false negative error may be reduced by controlling recovery, increasing the effective volume of water tested, or analyzing additional samples or subsamples.

The limit of detection for a sample is different from the method detection limit. It is standard practice to report as the limit of detection 100 times the reciprocal of the equivalent volume of sample analyzed. For example, if a 100-L sample of high quality water is concentrated, half of the concentrated sample is examined microscopically, and no oocysts are observed; the reported limit of detection would be 1 × 100/50 or 2 oocysts/100 L. The practice of reporting no detected parasites and the equivalent volume analyzed is necessary, but it does not sufficiently define the absence of parasites. Conditions such as excess turbidity that typically reduce the equivalent volume below the standard volume sampled are often associated with potential parasite contamination, especially in source water. Therefore, the absence of detected parasites in a sample should not be taken as conclusive evidence that parasites are absent from the source water. On the other hand, the positive identification of oocysts is strong evidence of their presence. While false positives can occur because of contamination or misidentification, the likelihood of false positives can be reduced to acceptable levels by analyst training and good laboratory practice. Only when gross contamination or analyst error has been found may measurement results be censored (i.e., not reported).

3.1.2.3 Identification

The ICR method does not identify species of *Giardia* and *Cryptosporidium*. Therefore, species that are not pathogenic to humans can be reported. Refinements of antibody preparations might reduce the potential for requiring enhanced treatment of water containing protozoa that do not actually pose a risk to humans.

As discussed in Section 3.1.1, there are many potential interferences that may confound identification of *Giardia* cysts and *Cryptosporidium* oocysts. The use of specific antibody staining is essential; otherwise, cysts and oocysts could easily be overlooked on a microscope slide crowded with yeast cells, non-pathogenic protozoa, pollen grains, or other debris. Excessive debris may also interfere with the antibody reaction, producing false negatives. False positives are also possible if the analyst is not sufficiently skilled to recognize auto-fluorescing or cross-reacting structures that are not *Giardia* or *Cryptosporidium*, such as algal cells and invertebrate eggs (Clancy et al. 1994, LeChevallier et al. 1991a).

There is a risk of false positive identification of *Cryptosporidium* oocysts if fluorescence is the only diagnostic feature used. Some non-pathogenic organisms auto-fluoresce under ultraviolet light, although this problem can be reduced by the addition of goat serum to coat the fluorescent organisms (Rodgers et al. 1995). Cross-reactivity of antibodies should not be a serious problem. Because commercially available fluorescent antibodies are carefully characterized, false positive identifications due to cross-reactions can be controlled [a partial list (September 1996) of commercially available antibodies to *Cryptosporidium* and their specificities is available on the Internet at the EPA microbiology site, http://www.epa.gov/microbes/]. To avoid false positives, fluorescent particles must be positively identified by the presence of microscopically observed morphologies characteristic of *Giardia* and *Cryptosporidium*.

False negatives can occur if the concentration of cysts or oocysts is low and the recovery is poor. Diagnostic intracellular structures can be damaged by treatment with chlorine compounds and perhaps other disinfectants, or by freezing, causing false negatives.

3.1.2.4 Infectivity and Viability

Viability is the capability of an organism to exhibit metabolic activity or response to biochemical stimulus suggesting that it is alive, but whether it is capable of completing the life cycle in a host or causing infection is unknown. Infectivity is the ability of an organism to complete its life cycle within a host, confirming infection of the host. Not all cysts and oocysts that are detected are viable, nor are all viable cysts and oocysts infectious (Black et al. 1996). A study of source water found that 12.8 percent of *Giardia* cysts had "viable type morphologies" and 32 percent of *Cryptosporidium* oocysts contained sporozoites, indicating potential viability (LeChevallier et al. 1991c). The ability of oocysts to encyst is closely correlated with infectivity of neonatal mice (Korich et al. 1997). Presumptive diagnostic methods such as uptake of vital stain and exclusion of stain by living cysts (Belosevic et al. 1997, Black et al. 1996, Brown et al. 1996, Campbell et al. 1992, Grimason et al. 1994, Jenkins et al. 1997, Korich et al. 1997, Taghi-Kilani et al. 1996) or gene amplification primed by messenger RNA (Abbaszadegan et al. 1997b, Stinear et al. 1996) appear to potentially indicate viability. *In vitro* infection of cell monolayers with oocysts produces infected foci that can be identified and quantitated by IFA (Slifko et al. 1997) or polymerase chain reaction (PCR) amplification of messenger RNA (Rochelle et al. 1997).

3.1.2.5 Interference by Turbidity

Turbidity can interfere with the sensitivity of analysis of *Giardia* and *Cryptosporidium* because particles clog the filters used to concentrate protozoa and it may not be possible to analyze a sufficient amount of sample to achieve a reliable result. In a sample of poor quality source water, high turbidity might limit the examination to a small equivalent volume, for example 0.5 L, unless additional analyst time is allocated to conduct microscopic analysis. If no oocysts were observed, the reported limit of detection would be 1 × 100/0.5 or 200 oocysts/100 L. Thus, turbidity, which may be very high after a storm, can have an enormous impact on the limit of detection for a particular sample.

3.2 SUMMARY

A single analytical method for protozoa has been approved by the ICR. This method includes filtering water samples through a yarn-wound polypropylene filter; eluting, clarifying, and concentrating the cysts and oocysts; staining with an indirect fluorescent antibody; and identifying cysts and oocysts by microscopy. *Giardia* cysts and *Cryptosporidium* oocysts are difficult to capture from water samples, and losses occur throughout the recovery and concentration steps. *Giardia* and *Cryptosporidium* are identified at the genus level, but neither species, viability, nor infectivity is determined. The ICR procedure is described in detail, and laboratories performing the analysis participate in a closely monitored quality control (QC) program, so results should be consistent among laboratories. The limit of detection of a sample depends on the quality of water being sampled, which affects the volume of water that can be analyzed. Debris in the water samples may increase the limit of detection by decreasing the equivalent volume that can be analyzed. The limit of detection for finished water can approach 0.1/100 L. However, the technique is difficult and time-consuming to perform, and analysts must have extensive training and experience.

4. SOURCES AND TRANSMISSION OF PATHOGENS

This chapter presents recently published information on sources of *Cryptosporidium* in the environment and evidence for waterborne and direct contact transmission of the parasite in human populations; the sources and transmission of *Giardia* are discussed to a lesser extent. The waterborne routes of cryptosporidiosis transmission are presented in Sect. 4.1, as are the observed efficiencies of water filtration systems in reducing the numbers of the pathogens in finished drinking water supplies. Direct contact transmission routes of cryptosporidiosis and to a lesser extent giardiasis are presented in Sect. 4.2.

Cryptosporidium oocysts and *Giardia* cysts are transmitted by the fecal-oral route, that is, oocysts excreted in the feces by an infected host are ingested or inhaled by another susceptible host. There have been numerous studies conducted to understand the sources, fate and transport, and source water occurrence and densities of *Cryptosporidium* and *Giardia* throughout the United States. Numerous factors affect the detection and recovery of *Cryptosporidium* from environmental samples such as oocyst age, coating of oocyst walls by fecal matter, sample collection methods, antibody stains, and microscopic interference due to particulate matter or organic debris. Because of these and other numerous factors, analysis is usually only performed on a small amount of the concentrated pellet after sample collection. The analysis of a portion of this pellet assumes a homogeneous distribution of oocysts in the sample and the environment and results in an elevated detection limit for that analysis. Because of this variation in recovery and detection limit and the number of samples necessary to capture extreme values, it is recommended that results given in this section be considered as a range of what has been detected and comparisons between data should be done with caution (i.e., one source is worse than another). Given the effects of the sample collection and matrices on recovery, it is important to note that trends in data must also be interpreted with caution because they may be due to analytical artifacts and not true mechanisms. Therefore, observation of the results of just one study would be improper and the aggregated results in numerous studies should be used to provide a range of potential protozoa occurrence and density. Also, given the acknowledged deficiencies of the method to detect and recover *Cryptosporidium* or *Giardia* in these environmental samples with elevated detection limits, the detection of protozoa is significant, because the "true" density values could potentially be greater than those measured.

The analytical method, unless otherwise mentioned for a specific study, did not have the ability to determine the viability, infectivity, or species of the protozoa detected. Until a better analytical method is available, any organism detected is usually conservatively considered viable and infective. Also, the analytical method described in the previous chapter was usually adapted for the various soil, fecal, sewage, and high turbidity source water matrices. This modified method still produced false positive and negative results and exhibited highly variable recoveries to an even greater extent than that measured in studies using low turbidity and interference matrices.

4.1 WATERBORNE TRANSMISSION

The waterborne transmission of cryptosporidiosis is well documented (Steiner et al. 1997, Craun 1996, Meinhardt et al. 1996). Outbreaks of cryptosporidiosis have been traced by public health officials to contaminated sources, including well water, treated drinking water supplies, or water accidentally contaminated and ingested at public recreational facilities such as swimming pools or water parks (Steiner et al. 1997). Sources of *Cryptosporidium* oocysts and routes of transmission are illustrated in Figure 4-1. This report focuses primarily on sources and transmission in water intended for drinking.

4.1.1 Sources of Drinking Water Contamination

It is commonly accepted that *Cryptosporidium* oocysts and *Giardia* cysts are ubiquitous at low levels in the environment. Sources of *Giardia* and *Cryptosporidium* associated with representative land use environments are summarized in Table 4-1 (*Giardia lamblia*) and Table 4-2 (*Cryptosporidium parvum*). Commonly identified sources of cysts and oocysts include raw and treated sewage, stormwater runoff from agricultural and urban areas, and recreational use of rivers and reservoirs used for surface water supplies.

Studies of North American watersheds have documented background levels of contamination by *Cryptosporidium* oocysts and *Giardia* cysts (Ongerth and Stibbs 1987, Rose 1988, Isaac-Renton et al. 1994, Hansen and Ongerth 1991, LeChevallier et al. 1991b, LeChevallier and Norton 1995). Oocyst and cyst concentrations in source waters and treated water have been shown to vary with human activities in the watershed and seasonal variations in storm water runoff and production (shedding) of oocysts by animals or human users of the watershed (Hansen and Ongerth 1991). Even protected watersheds (e.g., reservoirs where human activity is prohibited or limited) will typically contain a low concentration of oocysts and cysts because infected wild animals cannot be excluded from these areas (Hansen and Ongerth 1991).

Hansen and Ongerth (1991) examined the influence of watershed management activities on oocyst concentrations in river water samples during a 3-month study of adjacent watersheds. Influences of animal habitat were virtually indistinguishable in the watersheds, but human and associated domestic animal activity were markedly different from one location to another. Oocyst concentrations and production rates of a downstream area influenced by dairy farming were nearly 10 times higher than rates at upstream stations. The lowest oocyst concentrations (0.15 to 0.45 oocysts/L) were observed in a large, well-managed watershed where human activity was limited to a well-monitored lumber industry. The highest concentrations (10 to 60 oocysts/L) were observed at the sites farthest downstream, below numerous dairy farms, early in the sampling period when runoff influence was greatest; concentrations were significantly lower in dry periods. The authors concluded that human and domestic animal activities can make an overall 10-fold difference in the level of oocyst contamination in a watershed (Hansen and Ongerth 1991).

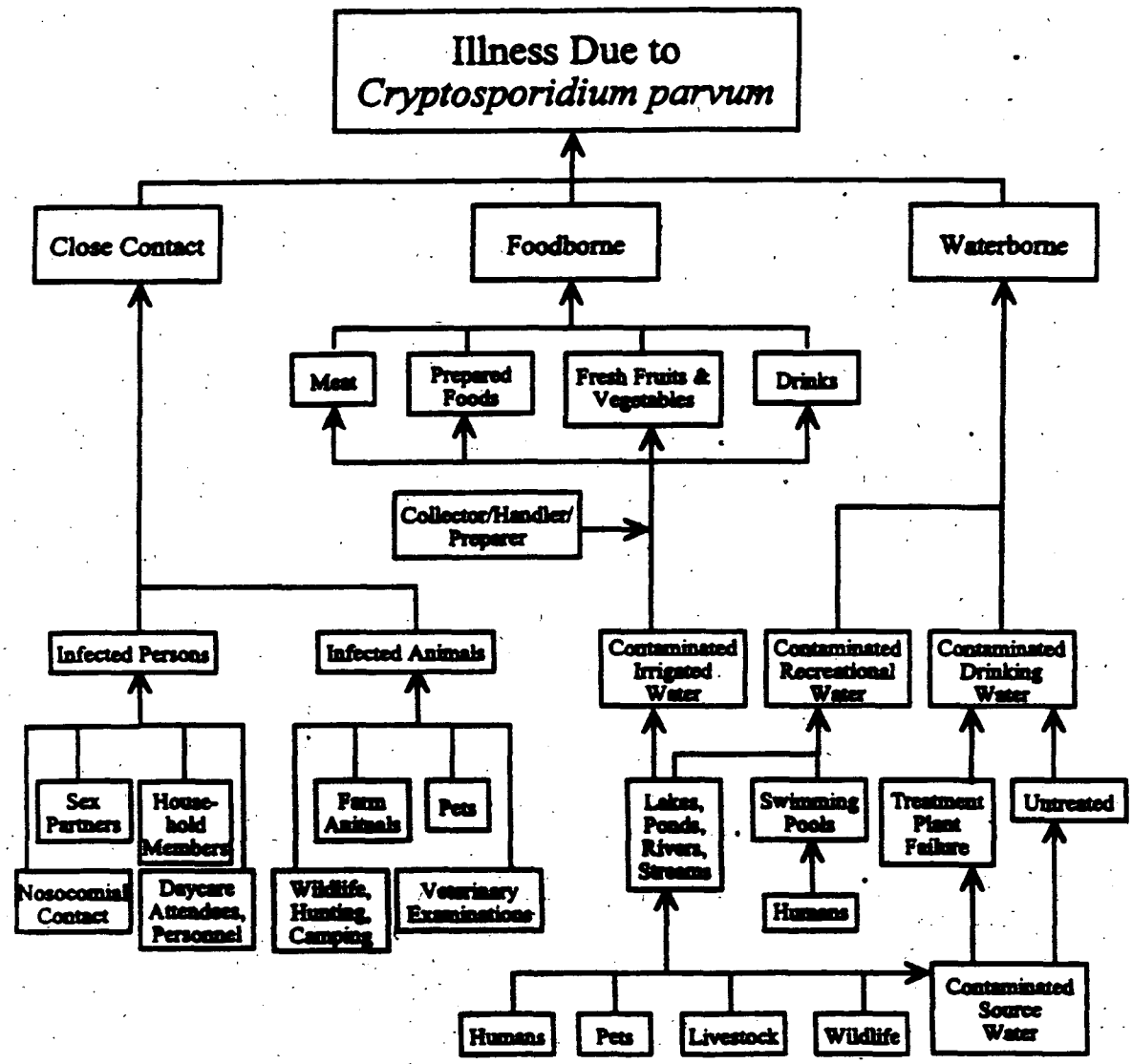


Figure 4-1. A "Fault Tree" Depicting Sources of Oocysts and Routes of Transmission.
***Source*: Adapted from Fayer et al. 1997, courtesy of Nancy N. Subat.**

Table 4-1. Sources of *Giardia lamblia* and their Discharge Concentrations

Land use	Source	Concentration	Reference
Parks/recreational	Beaver	Unknown	Erlandsen and Bemrick 1988
Parks/recreational	Muskrat	Unknown	Erlandsen and Bemrick 1988
Municipal/ residential	Human stool in raw sewage	3×10^6 cysts/person/day	Lin 1985
Municipal/ residential	STP effluents	13.2–1,347/100 L	Sykora et al. 1987
Municipal/ residential	Raw sewage influent	140–180 cysts/L	Casson et al. 1990
Municipal/ residential	STP effluent (activated sludge)	400 cysts/100 L geom. avg.	Casson et al. 1990
	STP effluent (trickling filter)	1100 cysts/100 L geom. avg.	
River	STP effluents	105.3–4613.6 cysts/100 L	States et al. 1995
Municipal/ residential	STP effluents	1801–29,349 cysts/100 L primary 0–2538 cysts/100 L secondary 0–1600 cysts/100 L tertiary	PADER 1995
Municipal/ residential	STP effluents	<61–1.2×10^4 cysts/100 L untreated 25–1.1×10^3 cysts/100 L secondary <1.3–13 cysts/100 L tertiary (sand filtration)	Rose et al. 1996
Water reclamation facility	Untreated wastewater	3.9×10^3 cysts/100 L (geometric mean)	Rose et al. 1996
Water reclamation facility	Treated water in storage tank	0.3 cysts/100 L (geometric mean)	Rose et al. 1996
Urban	Combined sewer overflow	2.8×10^4 cysts/100 L (geometric mean)	States et al. 1997
Urban	STP effluent	664 cysts/100 L (geometric mean)	States et al. 1997
Agricultural	Dairy farm runoff	82 oocysts/100 L (geometric mean)	States et al. 1997

Note: Additional unquantifiable sources that can shed *Giardia lamblia* are dogs, cats, coyotes, and cattle.
Source: Crockett and Haas 1995b.
STP = Sewage treatment plant.

States et al. (1997) monitored multiple sources of cyst and oocyst contamination to rivers supplying raw water to urban treatment facilities. Data were collected from dairy farm and sewage plant effluents, combined sewer overflows, and filter backwash water, as well as routine monitoring locations in the treatment facilities. They detected cysts and oocysts in over 50 percent of raw water (river) samples, and concluded that untreated sewage contamination and agricultural runoff from dairy farms were significant sources of parasites in raw water supplied to the utilities.

Table 4-2. Sources of *Cryptosporidium parvum* and their Discharge Concentrations

Land use	Source	Concentration	Reference
Municipal/urban	Raw sewage	5.18×10^3/100 L avg.	Madore et al. 1987
Municipal/urban	Treated sewage	1.3×10^3/100 L avg.	Madore et al. 1987
Municipal/ residential	STP (secondary treatment effluent)	5–17 oocysts/L	Musial et al. 1987
Agricultural	Calves, lambs	10^{10} oocysts/day for up to 14 days	Current and Garcia 1991
Municipal/urban	AIDS patient (infected)	6.0×10^6 to 1.2×10^9 oocysts/day	Goodgame et al. 1993
Agricultural	Calves (infected)	10^3 oocysts/g and deposit 5–15 kg feces/day	Breach et al. 1994
Agricultural	Cow (infected)	10^4 oocysts/g and deposit 25–30 kg feces/day	Breach et al. 1994
River	STP effluent	117.5–4927.2 oocysts/100 L	States et al. 1995
Water reclamation facility	Untreated wastewater	3.7×10^2 cysts/100 L (geometric mean)	Rose et al. 1996
Water reclamation facility	Treated water in storage tank	0.3 cysts/100 L (geometric mean)	Rose et al. 1996
Urban	Combined sewer overflow discharge	2,013 oocysts/100 L (geometric mean)	States et al. 1997
Urban	STP effluent	924 oocysts/100 L (geometric mean)	States et al. 1997
Agricultural	Dairy farm runoff	42 oocysts/100 L (geometric mean)	States et al. 1997

Note: Additional unquantifiable sources that can shed *Cryptosporidium* spp. are dogs, cats, and 40 other mammals.
Source: Crockett and Haas 1995b.

In another recent study, in British Columbia, Ong et al. (1996) reported higher frequency, density, and infectivity rates of both *Cryptosporidium* and *Giardia* in a watershed that had unrestricted cattle access to surface water in comparison with a watershed with restricted cattle access. Atwill et al. (1997) found that feral (wild) pigs that had unrestricted access to source water were a significant source of oocyst and cyst contamination to a watershed in California, the rate of oocyst shedding increasing with the density of the pig population.

Stormwater runoff is an important source of oocyst and cyst contamination of raw water in surface water supplies. LeChevalier et al. (1997a) and Stewart et al. (1997a) evaluated the effect of stormwater runoff on density of *Giardia* cysts and *Cryptosporidium* oocysts in surface water sources. They observed that the greatest densities of oocysts and cysts were detected during the "first flush" after a rainfall or during source water turbidity spikes. Potential sources of parasites in stormwater runoff to the two watersheds studied by Stewart et al. (1997a) included wildlife, wastewater facilities, residential development, recreational use, and livestock grazing. Of 20, 4-L grab samples collected during the first flush from storms, 12 (60 percent) were positive for cysts and 7 (35 percent) were positive for oocysts (Stewart et al. 1997a). Many of these samples would have been too turbid to have been sampled by conventional filter sampling apparatus (Stewart et al. 1997a).

Wastewater treatment systems or individual septic systems along rivers may discharge oocyst-contaminated fecal wastes into the source water of a downstream utility. In a recent EPA survey of water systems, 79 percent of water systems surveyed reported that septic systems located within 2 miles of source drinking water intakes are potential sources of raw water contamination, compared with 55 percent of systems reporting agricultural runoff occurring within 2 miles of their intakes (EPA 1997a). Other contributing sources of human and animal fecal waste potentially impacting source water quality are urban runoff (cited by 31 percent of water systems) and sewage discharge (cited by 27 percent of water systems) (EPA 1997a). A 1992 waterborne outbreak of cryptosporidiosis in Jackson County, Oregon, was attributed in part to excessive agricultural and sewage discharges contaminating the watershed serving the local treatment plant (Leland et al. 1993).

Because cryptosporidial infections in immunocompromised individuals can be chronic, their shedding of oocysts is a persistent source of the organisms in domestic sewage (Crockett and Haas 1997). Crockett and Haas (1997) estimated that a 10 mgd discharge of raw sewage to source waters is roughly equivalent to the daily loading (oocyst shedding) by 200 chronically ill, immunocompromised persons and the shedding of *Giardia* cysts by 100 immunocompetent persons. Shedding by calves and lambs presents an even greater threat to source water. The daily loading (oocyst shedding) by one infected calf or lamb can produce more oocysts than 1,000 immunocompromised persons (Crockett and Haas 1997). In a recent monitoring study designed to measure the relative contribution of heavy agricultural runoff to a creek feeding a river used as source water for an urban water purification facility, Crockett and Haas (1997) measured *Cryptosporidium* densities in the creek that were greater than those detected in 70-90 percent of all samples measured statewide during an equivalent year of monitoring; the highest *Giardia* cysts densities in the same creek were greater than 800 percent of those observed in water treatment systems without the heavy agricultural runoff (Crockett and Haas 1997).

In a 2-year study, States et al. (1997) monitored oocyst densities in three types of contamination sources: combined sewer overflow (CSO) discharges, sewage treatment plant effluent, and dairy farm runoff. The occurrence of oocysts in these sources was 80, 33, and 82 percent of samples, respectively. The highest mean density of oocysts in samples from this study was from the CSOs (geometric mean of 2,013 oocysts/100 L), followed by the sewage treatment plant (geometric mean of 924 oocysts/100 L), then the dairy farm (geometric mean of 42 oocysts/100 L (States et al. 1997).

In addition to contamination by storm water runoff, there is evidence that *C. parvum* from infected mammals could be ingested and deposited in other watersheds by birds as vectors (Graczyk et al. 1996). Fayer and associates (1997) showed that ducks and geese experimentally exposed to *Cryptosporidium* could act as mechanical vectors (carriers) for oocysts that remain infective for mice, even though the *C. parvum* was unable to establish infection in the waterfowl. In the same study, oocysts taken up by oysters remained infective for mice for one week after the oysters were first exposed. Graczyk et al. (1998) found oocysts in the tissues and feces of freshwater clams, *Corbicula flumenea*, exposed for 24 hr to water contaminated with 1×10^6 oocysts per liter. Oocysts extracted from the clam tissues 7 days following exposure were infectious for neonatal BALB/c mice. Smith et al. (1993) demonstrated that gulls (*Larus* sp.) could transport *Cryptosporidium* in their

fecal droppings. The likelihood of spreading viable, infective oocysts to susceptible species, including humans, is thus enhanced by the existence of such vectors.

The contribution of seasonal turnovers to cyst/oocyst levels in water supplies from lakes and reservoirs has not been documented. However, accidental human defecation during recreational use of source waters has been documented as a seasonal source of *Cryptosporidium* contamination in surface water supplies (Moore et al. 1993, Kramer et al. 1996). Stewart et al. 1997b modeled the impact of body-contact recreation on *Cryptosporidium* levels in a drinking water reservoir. Pathogen inputs were calculated based on projected recreator use data for the reservoir and available data from Yates et al. (1995) on the concentrations of pathogens in feces and shedding rates. Stewart et al. (1997b) estimated that body contact recreation in the reservoir would increase consumer risk by a factor of 20 to 140 times greater than background, with a maximum peak value of 2500 oocysts/100 L in the reservoir resulting in a 6.6×10^3 greater risk to the consumer. The model further demonstrated that adding body contact recreation to a formerly restricted reservoir would result in an increase of *Cryptosporidium*. The model was also able to predict the number of "body-contact recreators" that could be permitted without a need for additional water treatment.

4.1.2 Treatment and Removal

4.1.2.1 Cryptosporidium and Giardia Persistence and Viability during Treatment

A variety of factors influence the survival of oocysts, cysts, and other enteric pathogens. The persistence and viability of *Cryptosporidium* and *Giardia* in the environment are discussed in Sects. 2.1.5 and 2.2.5, respectively. Disinfection procedures are being sought to deal effectively with contamination of recreational and drinking water (Fayer et al. 1997).

Cordell and Adiss (1994) reviewed conditions of oocyst survival, noting that survival is decreased by extremes in temperature and by drying. Laboratory studies have shown that *Cryptosporidium* oocysts stored in containers that exclude air can remain viable for 8 to 9 months, but excystation seems to occur soon after exposure to air (Tzipori 1983). Although incidence of *Cryptosporidium* infection and contamination from aerosols is well documented (Blagburn and Current 1983, Hojlyng et al. 1987), the inactivation rate of *Cryptosporidium* oocysts in aerosols has not been determined.

Temperature is a key factor affecting survival (Rose 1997). Fayer and Nerad (1996), testing oocysts frozen at -10, -15, -20, and -70°C for 1 to 168 hours, demonstrated that oocysts of *C. parvum* in water can be both viable and infective after freezing but that oocysts survive longer at higher freezing temperatures. Freezing at -20°C for 72 hours (Robertson et al. 1992, Sherwood et al. 1982) or heating to 45 to 55°C for 20 minutes greatly reduces or eliminates infectivity. However, Robertson et al. (1992) noted that although desiccation is lethal, a small proportion of oocysts can survive for at least 775 hours at -22°C (oocysts remained viable, but infectivity is not known). Blewett (1989a) observed a 92 percent reduction in oocyst viability (assessed by excystation) following exposure to a temperature of 55°C for 5 minutes. Fayer et al. (1990) reported a decrease in oocyst infectivity after warming to 45°C for 5 to 20 minutes. Fayer (1994) evaluated the temperature limits of *Cryptosporidium* oocyst infectivity. The data indicated that when water

containing *C. parvum* oocysts reached temperatures of 72.4°C or higher within 1 minute or when the temperature was held at 64.2°C or higher for 2 minutes of a 5-minute heating cycle, infectivity was lost. Table 4-3 summarizes some results of temperature and other physical methods of disinfection on oocysts.

Table 4-3. Physical Disinfection of *Cryptosporidium* Oocysts

Agent	Conditions	Results	Test	Reference
Heat	121°C, 10 minutes	Protein changes	DEP	Archer et al. 1993
Heat	50–55°C, 5 minutes	NI	*In vivo*	Blewett 1989a
Heat	45°C, 20 minutes	NI	*In vivo*	Anderson 1985
	60°C, 6 minutes	NI	*In vivo*	
Heat	59.7°C, 5 minutes	I	*In vivo*	Fayer 1994
	64.2°C, 5 minutes	NI	*In vivo*	
	67.5°C, 1 minute	I	*In vivo*	
	72.4°C, 1 minute	NI	*In vivo*	
Freezing	-196°C, 10 minutes	NI	*In vivo*	Sherwood et al. 1982
	-20°C, 3 days	NI	*In vivo*	
Freezing	-70°C, 1 hour	NI	*In vivo*	Fayer and Nerad 1996
	-20°C, 8 hours; 1 day	I; NI	*In vivo*	
	-15°C, 24 hours; 1 week	I; NI	*In vivo*	
	-10°C, 1 week	I	*In vivo*	
Freezing	Liquid nitrogen	100% reduced	Ex/dyes	Robertson et al. 1992
	-22°C, ≤32 days	98% reduced	Ex/dyes	
Ultraviolet	15,000 mW/sec for 2 hours	I	*In vivo*	Lorenzo-Lorenzo et al. 1993
	15,000 mW/sec for 2.5 hours	NI	*In vivo*	
Ultraviolet	80 mW/sec cm^{-2}	90% reduced	Ex	Ransome et al.1993
	120 mW/sec cm^{-2}	99% reduced	Ex	
Ultraviolet	8748 mW/sec cm^{-2}	100% reduced	Ex/dyes	Campbell et al. 1995
Pulsed light	1 J/cm^2	100% reduced	*In vivo*	Dunn et al. 1995
Drying	Air dried, 2 hours	97% reduced	Ex/dyes	Robertson et al. 1992
	Air dried, 4 hours	100% reduced	Ex/dyes	
Drying	Air dried in feces, 1–4 days	NI	*In vivo*	Anderson 1986

Source: Fayer et al. 1997.

In vivo testing performed in mice.

DEP = Dielectrophoresis I = Infectious

Ex = Excystation NI = Noninfectious

Laboratory evaluation of thermophilic (55°C) aerobic digestion of sludge and sludge pasteurization at 55°C showed that sludge treatment at this temperature inactivated *Cryptosporidium* oocysts (Whitmore and Robertson 1995).

Testing the effect of pasteurization on infectivity of oocysts in water and milk, Harp et al. (1996) confirmed that high-temperature (71.7°C), short-time (5 to 15 seconds) pasteurization is sufficient to destroy the infectivity of *C. parvum* oocysts in water and milk.

Air drying aqueous suspensions of oocysts at room temperature for 4 hours eliminated viability (Robertson et al. 1992), but oocysts in fecal material are protected from desiccation and thus their viability in the environment is prolonged (Rose 1997).

Numerous studies (Cordell and Adiss 1994, Fayer et al. 1997) have shown that many disinfectants have little, if any, effect on oocyst viability or infectivity. Table 4-4 summarizes some results of halogen disinfection of *Cryptosporidium* oocysts. Chlorine and sodium hypochlorite (chlorine bleach) in typically used concentrations are poor disinfectants for *Cryptosporidium*, although full strength bleach (5.25 percent sodium hypochlorite) destroyed oocyst infectivity to mice after 10 minutes (Current 1986). Recent research on chlorine dioxide inactivation of *Cryptosporidium* has demonstrated moderate effectiveness at feasible concentrations (considering disinfection byproducts) and reasonable contact times. LeChevallier et al. (1996) showed that chlorine dioxide was moderately successful at disinfecting *Cryptosporidium*. They achieved up to about 1.3 log of disinfection at pH 6 when the treatment was carried out at 20°C; less than 1 log if disinfection occurred at pH 8 or when the treatment was done at 10°C. Disinfection effectiveness decreases with declining temperature. Finch et al. (1998) investigated temperature effects on *Cryptosporidium* inactivation at pH 6 and showed that the contact time for 1-log inactivation at a residual of 1 mg/L increased from 74 minutes at 25°C to 311 minutes at 1°C. Of all the disinfectants used in water treatment plants, ozone is the most effective in inactivating *Cryptosporidium*; i.e., significant levels of inactivation are achieved at low disinfectant residual concentrations and short (less than 5 minutes) contact times; however, disinfection effectiveness decreases with increasing pH. Finch et al. (1997) reported 1.5 logs inactivation at 22°C and pH 6 and 0.5 logs at pH 8. Jenkins et al. (1998) determined the effects of increasing free ammonia concentrations and length of exposure on oocyst viability. At a constant temperature of 24°C, increasing exposure times from 10 minutes to 24 hours and increasing the concentration of ammonia increased the inactivation rate of wild-type oocysts. Oocysts exposed to ammonia at 24°C are inactivated more quickly than at 4°C. Results indicated that pH associated with various concentrations of ammonia was not a factor in oocyst inactivation, but pH did tend to affect the permeability of oocyst walls (Jenkins et al. 1998). Although oocysts survive standard chlorine disinfection treatment, laboratory studies have shown that the application of chlorine or ozone followed sequentially by monochloramine inactivates *Cryptosporidium* (Newman 1995).

Korich et al. (1990) exposed *Cryptosporidium parvum* to ozone, chlorine dioxide, chlorine, and monochloramine and tested viability using a comparison of excystation and mouse infectivity. Ozone and chlorine dioxide inactivated oocysts more effectively than did chlorine and monochloramine. Ozone (1 ppm

Table 4-4. Halogen Disinfectants Tested Against *Cryptosporidium* Oocysts

Disinfectants	Conditions	Results	Test	Reference
Bromine	1180 mg/L, 60 minutes	88.5% reduction	Excyst	Ransome et al. 1993
Bromomethane	100% gas, 24 hours	NI	*In vivo*	Fayer et al. 1996a
Chlorine	5 mg/L	NR	Excyst	Quinn and Betts 1993
Chlorine	80 mg/L, 2 hours	99% reduction	*In vivo*	
Chlorine	867–5118 mg/L, 24 hours	72.5% to 88.1% reduction	Excyst	Ransome et al. 1993
Chlorine	16,000 mg/L, 12 hours 28,000 mg/L, 24 hours	90% reduction 100% reduction	Excyst	Smith et al. 1990
Chlorine	80 ppm, 90 minutes	90% reduction	Excyst	Korich et al. 1990
Chlorine after shaking with sand	1 mg/L, 5 minutes, 25°C	Reduction	Excyst	Parker and Smith 1993
Hypochlorite	2.8%, 30 minutes, 25°C	89% reduction	Excyst	Sundermann et al. 1987
Hypochlorite	1%, 30 minutes, 22°C 1%, 30 minutes, 37°C	55% reduction 69% reduction	Excyst	Blewett 1989a
Hypochlorite	3%, 18 hours	I	*In vivo*	Campbell et al. 1982
Hypochlorite	5.25%, 2 hours, 20°C	I	*In vivo*	Fayer 1995
Chlorine dioxide	0.007 mg/L, 16 minutes 0.22 mg/L, 30 minutes	97% reduction 94.3% reduction	Excyst *In vivo*	Peeters et al. 1989
Chlorine dioxide	0.6 mg/L, 90 minutes	90% reduction	*In vivo*	
Chlorine dioxide	1.3 mg/L, 1 hour	92.7% reduction	Excyst	Korich et al. 1990
Chlorine dioxide	4.03 mg/L, 15 minutes	96% reduction	Excyst	Ransome et al. 1993
Chlorine dioxide (Exspor)	Conc. not given, 30 minutes, 22°C	95% reduction	Excyst	Blewett 1989a
Chloramine	3%, 24 hours	I	*In vivo*	Pavlasek 1984
Monochloramine	80 ppm, 90 minutes	90% reduction	Excyst	Korich et al. 1990
Monochloramine	0.066 mg/L, 48 hours 3.76 mg/L, 24 hours	80.5% reduction 76.8% reduction	Excyst	Ransome et al. 1993
Monochloramine	80 mg/L, 90 minutes	90% reduction	Excyst	
Iodophore	4%, 18 hours	I	*In vivo*	Campbell et al. 1982
FAM 30 (Iodophore)	1%, 30 minutes, 22°C 1%, 30 minutes, 37°C	20% reduction 90% reduction	Excyst	Blewett 1989a

Table 4-4 (continued)

Disinfectants	Conditions	Results	Test	Reference
Provid (Iodine)	10%, 30 minutes, 22°C 10%, 30 minutes, 37°C	19% reduction 53% reduction	Excyst	Blewett 1989a
Iodine	10 mg/L, pH 4–7, 1 hour	84.5% to 56.4% reduction	Excyst	Ransome et al. 1993
Iodine bromide	39.1 mg/L, 60 minutes	71.7% reduction	Excyst	Ransome et al. 1993
Mixed-oxidant solution	5 mg/L, 4 or 24 hours	99.9% inactivation	*In vivo*	Venczel et al. 1997

Source: Fayer et al. 1997.
Excyst = Excystation.
I = Infectious.
NI = Noninfectious.
NR = No marked reduction.

or 1 mg/L) for 5 minutes produced greater than 90 percent inactivation. Chlorine dioxide (1.3 ppm) inactivated 90 percent after 1 hour, and chlorine (80 ppm) and monochloramine (80 ppm) required 90 minutes for 90 percent inactivation. Results indicated that *C. parvum* oocysts are 30 times more resistant to ozone and 14 times more resistant to chlorine dioxide than *Giardia* cysts exposed to the same disinfectant under the same conditions (Korich et al. 1990).

Oppenheimer et al. (1997) reported on the relationship between *Cryptosporidium* oocyst inactivation and product (CT) of disinfectant residual (C) and disinfectant contact time (T) for alternative disinfectants applied to a wide range of source waters. The disinfectants investigated included ozone, addition of chlorine followed by chlorine, addition of chlorine followed by chloramines, or addition of ozone followed by chloramines. The data suggest no appreciable biocidal effect, either for chlorine alone or for chlorine followed by chlorine. No more than 0.5-log inactivation was achieved using chlorine followed by chloramines, even at impracticably high CT levels. A range of ozone CT values (calculated or simulated) was tested for target levels of inactivation at different temperatures. It should be noted that the temperature-adjusted simulated CT values for ozone inactivation of oocysts were 5 to 20 times the CT values listed for inactivation of *Giardia* cysts in the Surface Water Treatment Rule Guidance Manual (Oppenheimer et al. 1997). Some enhancement of inactivation was observed if the ozonated sample was immediately followed by chloramination (Oppenheimer et al. 1997).

Finch et al. (1997) evaluated the effects of several different disinfection methods on the inactivation of *Cryptosporidium*, noting that chlorine and monochloramine alone at practical plant levels are not effective, ozone and chlorine dioxide have been described as the most promising alternative disinfectants, and chemical disinfectants used sequentially exhibit synergistic effects that produce significantly more oocyst inactivation at pH 6 and 8, but not at higher pH. Finch et al. (1997) discuss problems with interstudy comparisons of oocyst disinfection, study reproducibility, and possible sources of error, including underestimation of inactivation.

Some of the problems, such as those associated with oocyst viability measurement, are addressed in Sect. 3.1.2.4.

Venczel et al. (1997) evaluated an electrolytically produced mixed-oxidant solution (containing free chlorine, chlorine dioxide, ozone, hydrogen peroxide, and other short-lived oxidants) for inactivating *Cryptosporidium parvum* oocysts. The disinfection efficacy of the mixed-oxidant solution was compared with that of free chlorine on the basis of equal weight per volume concentrations of total oxidants. The mixed-oxidant solution was considerably more effective than free chlorine, with a 5-mg/L dose of mixed oxidants producing a greater than 3-log (greater than 99.9 percent) inactivation of *C. parvum* oocysts (Iowa strain) in 4 hours. Free chlorine produced no measurable inactivation by 4 or 24 hours (Venczel et al. 1997).

The survival pattern of oocysts suggests that once an initial contamination event has occurred, water can remain a source of viable oocysts for days (Heisz 1997, Lisle and Rose 1995). Evidence for this is pronounced (Sect. 2.1.5), particularly the 176 days to produce die-off rates of 96 percent in tap water and 94 percent in river water (Lisle and Rose 1995, Robertson et al. 1992). After 2 days, a realistic contact time in most water distribution systems, only 37 percent of the oocysts became nonviable (Lisle and Rose 1995).

Simple disinfection has been inadequate as the only treatment of surface water, prompting researchers to test the effects of various disinfection regimes on *Giardia* cysts. Isaac-Renton et al. (1996) determined that although *Giardia* cyst concentrations were lower in chlorinated drinking water samples than in raw water samples, viability of cysts was decreased but not eliminated. Craun (1990) reported giardiasis outbreaks occurred at a rate of 24.4 waterborne outbreaks/1000 water facilities in United States communities in which surface water was treated only with chlorine. Jarroll (1988) and Jakubowski (1990) reviewed *Giardia* cyst disinfection. Some of those data, as well as additional studies, are summarized below and in Table 4-5.

A study by Jarroll et al. (1981) indicated the important interrelationships between temperature, pH, and contact time on the effects of chlorine concentration on cyst viability. Using excystation as the criterion of cyst viability, these authors found that cyst survival in the presence of chlorine generally increased as the buffer pH increased. At 25°C, exposure to 1.5 mg chlorine/L for 10 minutes killed all cysts at pH 6, 7, and 8. At 15°C, 2.5 mg chlorine/L for 10 minutes killed all cysts at pH 6, but at pH 7 and 8 small numbers of cysts remained viable after 30 minutes but not after 60 minutes. At 5°C, 1 mg chlorine/L for 60 minutes failed to kill all the cysts at any pH tested. Additional test data indicated that low water temperatures required relatively high chlorine concentrations and long contact times to kill the cysts.

Hoffman et al. (1995) reported data from disinfection experiments with chlorine and chloramines, as well as tests with the major disinfection byproducts (chloroform, dichloroacetic acids, and trichloroacetic acids). Results indicated that chloramines required much higher contact time values than free chlorine. Also, the data indicated that chlorine and chloramine inactivation was more efficient at pH 6.5 than at 8.5, and at 25°C than at 5°C.

Table 4-5. Disinfectants Tested Against *Giardia* Cysts

Disinfectant	Conditions	Results	Viability assay	Reference
Chlorine	1 mg chlorine/L at 5°C for 10 minutes	35% (at pH 6) to 56% (at pH 8) cyst (*G. lamblia*) survival	Excystation	Jarroll et al. 1981
	2 mg chlorine/L at 5°C for 60 minutes	No cyst survival		
	2.5 mg chlorine/L at 15°C for 10 minutes	No cyst survival at pH 6, but 1.8% survival at pH 7		
	1.5 mg chlorine/L at 25°C for 10 minutes	No cyst survival		
Ozone	0.15 mg ozone/L for 0.97 minute at 25°C, or 0.48 mg ozone/L for 0.95 minute at 5°C	99% inactivation of *G. lamblia* cysts	Excystation	Wickramanayake et al. 1985
	0.18 mg ozone/L for 1.3 minutes at 25°C, or 0.70 mg ozone/L for 2.5 minutes at 5°C	99% inactivation of *G. muris* cysts		
Chlorine	0.3 to 2.5 mg chlorine/L at 0.5°C to 5.0°C at pH 6 to pH 8	Mean CT (chlorine concentration × time) to produce 99.9% to 99.99% inactivation: 185 to 280	Mongolian gerbil infectivity	Hibler et al. 1987
		To produce >99.99% inactivation: 220 to 290		
Ultraviolet irradiation	63,000 μW-sec/cm²	Less than 1-$\log_{10}$ reduction in cyst survival	Modified *in vitro* excystation	Rice and Hoff 1981

This trend was also evident in the results of a study to determine chlorine concentration and time (CT) required to inactivate *Giardia* cysts at 0.5°C to 5.0°C (Hibler et al. 1987). Higher CT values are required to inactivate cysts at temperatures between 0.5 and 5.0°C. than at temperatures above 5.0°C. There is also loss of biocidal efficiency at pH above 7.5. CT values were determined for final chlorine concentrations of 0.3 mg/L to 2.5 mg/L, and increasing chlorine concentrations above 2.5 mg/L is not recommended.

Ozone inactivation of *Giardia* cysts proved to be much more efficient than chlorine based on a contact time comparison with Jarroll et al. (1981) results, according to Wickramanayake et al. (1985). The reported

contact-time products of ozone at pH 7 to achieve 99 percent inactivation were 0.53 and 0.17 mg–min/L at 5°C and 25°C, respectively. Labatink et al. (1991) and Owens et al. (1994) also reported that ozone effectively inactivates *Giardia muris* cysts. The Owens et al. (1994) data indicated that the *G. muris* protozoa were 10 times more susceptible to ozone than *Cryptosporidium parvum*, which were also tested. Finch et al. (1993) reported comparative data for cyst inactivation by ozone for *G. muris* and *G. lamblia*, noting that the two species were not significantly different in their sensitivity to inactivation by ozone.

A study by Rice and Hoff (1981) of cyst inactivation by ultraviolet light showed that *G. lamblia* cysts have marked resistance to high doses of ultraviolet irradiation. Cyst viability was determined by a modified in vitro excystation procedure. The dose of ultraviolet irradiation that exhibited any inactivation of cysts (63,000 μW-s/cm^2) was far beyond the minimum dosage recommended by the U.S. Public Health Service for ultraviolet disinfection of water and maximum designed dose ranges for commercial manufacturers. The high doses produced less than a one-log reduction in cyst survival, suggesting that ultraviolet irradiation at conventional doses is not a viable alternative method of water disinfection when *G. lamblia* is present.

4.1.2.2 Removal in Treatment Systems

The contamination of watersheds and source water by *Cryptosporidium* and *Giardia* requires efficient removal in treatment systems to protect consumers from drinking water contamination. Because *Cryptosporidium* oocysts are resistant to inactivation by chlorine (the most commonly used disinfectant in the United States) and because of health concerns associated with high concentrations of disinfection by-products, recent studies have focused on predicting and improving rates of removal by filtration.

The typical treatment process to remove particles, including *Cryptosporidium*, from the raw water entails adding coagulating chemicals (e.g., alum) to the water so that the particles coalesce to form flocs that will either settle to the bottom of a sedimentation basin or will be removed through subsequent filtration. Numerous studies have been conducted evaluating the treatment conditions under which optimal removal of *Cryptosporidium* and *Cryptosporidium*-sized particles can be achieved. Aspects of *Cryptosporidium* removal and the results of those studies are discussed in this section.

Surface Water Treatment Configurations

There are several types of existing treatment systems for removing turbidity and microbial contaminants from raw water supplies. These systems include conventional treatment, direct filtration, slow-sand filtration, diatomaceous earth filtration, and membrane filtration. General descriptions of these treatment configurations follow.

Conventional treatment is the most widely used technology for removing turbidity and microbial contaminants from surface water supplies. Conventional treatment includes the pretreatment steps of chemical coagulation, rapid mixing, flocculation, and sedimentation, followed by filtration and disinfection. The filters used may be classified as either sand, dual-media (anthracite-sand), or tri-media, in which a third finer and heavier sand layer is used (EPA 1998d).

Direct filtration can consist of any one of several different process trains depending upon the application. In its simplest form the process includes only dual- or mixed-media filters (often pressure units) preceded by chemical coagulant application and mixing. Direct filtration is distinguished from conventional treatment by the fact that the sedimentation step is omitted, and the coagulated water proceeds directly to the filters. Mixing requirements can be satisfied by influent pipeline turbulence, although in large plants with gravity filters, an open rapid-mix basin with mechanical mixers is typically used. Raw water must be of seasonably uniform quality with turbidities routinely less than 5 NTU to be effectively filtered by a direct filtration systems. Variations of the process include either flocculation or a 1-hour contact basin step prior to filtration (EPA 1998d).

Slow-sand filtration is used principally in small communities that have a protected surface watershed and use only chlorination, and where raw water is of high quality (NTU less than 10). Filter sand depth ranges up to 42 inches, and the rate of filtration varies from 1 to 10 million gallons per acre per day (mgad), with 3 to 6 mgad (0.05 to 0.1 gpm/ft^2) the usual range. Many slow-sand filters have no pre-treatment, while others are preceded by coagulation, settling , or roughing filters. In addition to their slower flow rates, slow-sand filters also have smaller pore sizes between particles and function using both physical and biological mechanisms (EPA 1998d). For these reasons, slow sand filters may have larger acreage requirements than other filtration systems.

Diatomaceous earth (DE) filtration, also known as precoat or diatomite filtration, is applicable to direct treatment of surface waters for removal of relatively low levels of turbidity. Developed by U.S. military forces during World War II to remove cysts of *Entamoeba histolytica* from water, diatomite filters were later applied to civilian use, principally in the filtration of swimming pool water, and a few were constructed to treat municipal water supplies. Diatomite filters consist of a layer of DE approximately 1/8-inch-thick supported on a septum or filter element, supplemented by a continuous-body feed of diatomite to maintain porosity of the filter cake. The problems inherent in maintaining a perfect film of DE between filtered and unfiltered water, however, have restricted the use of diatomite filters for municipal purposes, except under certain favorable conditions (EPA 1998d).

Membrane filtration is a relatively new and growing technology in municipal drinking water treatment for providing barriers to turbidity, microorganisms, and many other water-borne contaminants. Pressure-driven membrane processes are usually the most appropriate for municipal water treatment and fall into four categories based on the size of the largest particle, colloid, or molecule that can pass through the membrane. The greater the removal capabilities of a membrane, the "tighter" it is said to be; generally the tighter the membrane, the higher the pressure required to filter water through it. The four categories of pressure-driven membranes, in order of increasing tightness, are as follows:

- *microfiltration* (MF) membranes remove particles at the micron or submicron levels, and can remove suspended matter and some colloidal material;

- *ultrafiltration* (UF) is capable of separating macromolecular materials, and can remove most microbial contaminants, except viruses, and some natural organic matter;

- *nanofiltration* (NF) can remove a high percentage of hardness ions as well as virtually all microbial contaminants; and

- *reverse osmosis* (RO) membranes can remove virtually all ionic species at high pressures.

Membrane systems can be used either as an addition to conventional treatment or alone. In conventional treatment systems, membranes are generally installed downstream of the conventional filters. When installed alone, membrane systems typically require prefiltration, pretreatment, and posttreatment steps (EPA 1998d).

Evaluating Treatment Performance

Treatment Efficiency. The effectiveness of removal and inactivation processes for *Cryptosporidium* is evaluated using "challenge" test conditions in bench- and pilot-scale applications. To test the efficiencies of a treatment process, large numbers of oocysts of a known quantity are added to the raw water so that the oocysts remaining following treatment can be measured (Frey et al. 1997). *Cryptosporidium* treatment removal efficiency is then calculated using the difference between the oocyst concentrations in the raw and finished waters. Removal efficiencies are typically expressed either in terms of percent removal or log removal as follows:

$$\% \text{ Removal Efficiency} = \frac{(\text{Raw Total Oocysts} - \text{Finished Total Oocysts}) \times 100\%}{\text{Raw Total Oocysts}}$$

$$\text{Log Removal Efficiency} = \text{Log}_{10}(\text{Raw Total Oocysts}) - \text{Log}_{10}(\text{Finished Total Oocysts})$$

The performance of a water treatment process for *Cryptosporidium* control must be challenged to the degree of its ability to remove or inactivate the oocysts. For example, if a technology can remove 10 oocysts, adding only 9 oocysts to the raw water to "challenge" the technology will not generate conclusive evidence that the technology could remove 10 organisms. Therefore, challenge tests are typically designed to capture several orders of magnitude of treatment performance so that the full range of a technology's performance can be measured (Frey et al. 1997).

For convenience in describing the multiple orders-of-magnitude used in evaluating treatment performance, the removal of waterborne organisms is often expressed on the base of a logarithmic (log) scale. For example, a log removal of 1.0 indicates a 90 percent reduction in oocyst densities (e.g., from 1000 to 100 oocysts/L); 2 log removal means that 99 percent is removed (e.g., from 1000 to 10 oocysts/L); 3 log removal means that 99.9 percent has been removed (e.g., from 1000 to 1 oocysts/L); and so on (SAIC 1998).

Particle Counting. Particle counting methods are used to quantify removal efficiencies of a system. The validity of using log reduction information from particle counters as a surrogate for the equivalent reduction in *Giardia* cysts and *Cryptosporidium* oocysts has been the subject of several studies. The ability of particle counters to accurately size *Giardia* cysts (7-11 mm) and *Cryptosporidium* oocysts (4-7 mm) has also been

investigated. Such studies have indicated that particle log removal evaluated within size ranges coincidental to the size of the organisms can be indicative of microorganism log removal, although the counters may tend to undersize the organisms. A recent study (O'Shaughnessy et al. 1997) evaluated particle counters used both to assess the removal efficiency of a treatment facility and as a process monitoring tool. These studies compared total counts and removal efficiency measured with a particle in relation to similar information obtained by scanning electron microscopy(SEM) and by microscopic particulate analysis (MPA), and also compared the accuracy and resolution of forward-angle light scatter (FALS) and light obscuration (LO) sensors in detecting microorganisms and latex spheres of known diameter. Log removal values were found to be comparable among the particle counting methods, and did not vary significantly across various size ranges of a FALS sensor. However, microorganisms were undersized by both sensor types when compared with sizes determined with an optical microscope. The results suggest that although counters may accurately indicate facility particle removal efficiency, counts made by a particle counter within a specific size range should be interpreted with caution. Total microorganism counts made by a particle counter should be viewed relative to previous counts and not as an absolute indication of the number of particles with diameters associated with that size range (O'Shaughnessy et al.1997)..

Pilot Plant Studies. Pilot plants are small-scale versions of a treatment process or system that are constructed to mimic the full-scale system and to allow control, monitoring, evaluation, and change of process variables as needed. In order for pilot plant study results to be meaningful, however, the pilot plants must be properly designed and the influence of various operational factors on the subsequent results must be understood and controlled (McTigue and MacPhee 1997). For example, differences in removal measured from one study to the next could be due to the concentration and way the spike dose was fed, system demand, oocyst age and preparation, and methods of sample collection and analysis. These factors can all cause significant over- or underestimation of the removal of oocysts in plant spiking studies. Important operational considerations in pathogen removal pilot plant studies include:

- *Pathogen Feed Method*—Cysts may be fed into the system by either a step dose or slug dose. Step dosing is generally accepted as the most appropriate feed method since a mass balance is achieved at the inlet and outlet of the plant, thus permitting comparisons of results between trials. Slug dosing would require collection of all cysts in the effluent to compute mass removal, which would be impossible in practice (McTigue and MacPhee 1997).

- *Pathogen Feed Concentration*—The feed solution must contain sufficient concentration of pathogens to permit log reduction calculations. This requires a direct microscopic count of the concentration of the commercially obtained feed stock prior to spiking, knowledge of the analytical detection limits, and calculation of the theoretical maximum log reduction that can be determined given the feed concentration and detection limits (McTigue and MacPhee 1997).

- *Quantity of Pathogen Feed Solution*—For step-dosing the system, the pathogen feed is started and allowed to flow into the system for a period equal to three detention times before sample collection. Enough pathogen feed solution would have to be prepared to last the required length of time at the flow rate of the pathogen feed pump (McTigue and MacPhee 1997).

- *Sample Collection Method and Enumeration*—The 1-micron nominal porosity cartridge sampling approved by ASTM recovers only a fraction of the pathogens present in a water sample; higher cyst recoveries are associated with the 3-micron membrane sampling method. On a percent recovery basis, the membrane sampling technique consistently recovers more cysts; on a log reduction basis, the two methods were similar, typically differing by less than 0.5 logs. Based on percent recovery data, however, the cartridge method has the potential for missing very low cyst concentrations, while the memorane technique has the potential for over-counting the pathogens (McTigue and MacPhee 1997).

Studies of Pathogen Removal Efficiencies

Research pertinent to *Cryptosporidium* and *Giardia* removal efficiencies are summarized in Table 4-6. Brief descriptions of these studies follow.

In a 1995 conventional treatment pilot plant study, raw water turbidities were between 0.2 and 13 NTU, and oocyst/cyst concentrations were between 10 and 200/L (Patania et al. 1995). When treatment conditions were optimized for turbidity removal at four different sites, *Cryptosporidium* removal ranged from 4.2 to 5.2 logs and *Giardia* removal ranged from 4.1 to 5.1 logs during stable filter operation. The median turbidity removal was 1.4 log, whereas the median particle removal was 2 logs. Median oocyst and cyst removal was 4.2 logs. A filter effluent turbidity of 0.1 NTU or less resulted in the most effective cyst removal. An increase in filter effluent turbidity to a range of 0.1 to 0.3 NTU reduced cyst removal by up to 1 log (see Figures 4-2 and 4-3). *Cryptosporidium* removal rates of less than 2.0 logs (indicated in Figures 4-2 and 4-3) occurred at the end of the filtration cycle.

Blackened data points in these figures represent data in which oocysts were not detected in the filtered water. The log removal values shown would be greater than indicated had the influent oocyst concentration been sufficiently high to show oocyst detection in the filtered water, or if the detection method for finished water has a low efficiency of recovery. The researchers also noted that removal of *Cryptosporidium* was 0.4 to 0.9 logs lower during filter maturation than during filter operation; *Giardia* removal was generally 0.4 to 0.5 logs lower during maturation (Patania et al. 1995). The removal of *Cryptosporidium* was higher for conventional treatment (including sedimentation) as compared to direct filtration. *Giardia* removal was also higher in conventional treatment. Figures 4-2 and 4-3 show aggregate pilot plant data from Patania et al. (1995).

Table 4-6. *Cryptosporidium* and *Giardia lamblia* Removal Efficiencies

Type of treatment plant	Log removal		Experimental design	Reference
Conventional filtration plants	*Cryptosporidium* *Giardia*	4.2–5.2 4.1–5.1	Pilot plants	Patania et al. 1995
	Cryptosporidium *Giardia*	1.9–4.0 2.2–3.9	Pilot-scale plants	Nieminski and Ongerth 1995
	Cryptosporidium *Giardia*	1.9–2.8 2.8–3.7	Full-scale plants	Nieminski and Ongerth 1995
	Cryptosporidium *Giardia*	2.3–2.5 2.2–2.8	Full-scale plants	LeChevallier and Norton 1992
	Cryptosporidium	2–3	Pilot plants	Foundation for Water Research, Britain 1994
	Cryptosporidium *Giardia*	1.5–2 1.5–2	Full-scale plant (operation considered not optimized)	Kelley et al. 1995
Direct filtration plants	*Cryptosporidium* *Giardia*	2.7–5.9 3.4–5.0	Pilot plants	Patania et al. 1995
	Cryptosporidium *Giardia*	2.7–3.1 3.1–3.5	Pilot plants	Ongerth and Pecoraro 1995
	Cryptosporidium *Giardia*	1.3–3.8 2.9–4.0	Pilot plants	Nieminski and Ongerth 1995
	Cryptosporidium	2–3	Pilot plants	West et al. 1994
Slow sand	*Cryptosporidium* *Giardia*	>3 >3	Pilot plant at 4.5 to 16.5°C	Schuler and Ghosh 1991
	Cryptosporidium	4.5	Full-scale plant	Timms et al. 1995
Diatomaceous earth	*Cryptosporidium* *Giardia*	>3 >3	Pilot plant; addition of coagulant increased removal beyond values shown	Schuler and Ghosh 1990
Microfiltration	*Cryptosporidium* *Giardia*	6.1–6.9 6–7	Pilot plant	Jacangelo et al. 1995

Source: EPA 1998d.

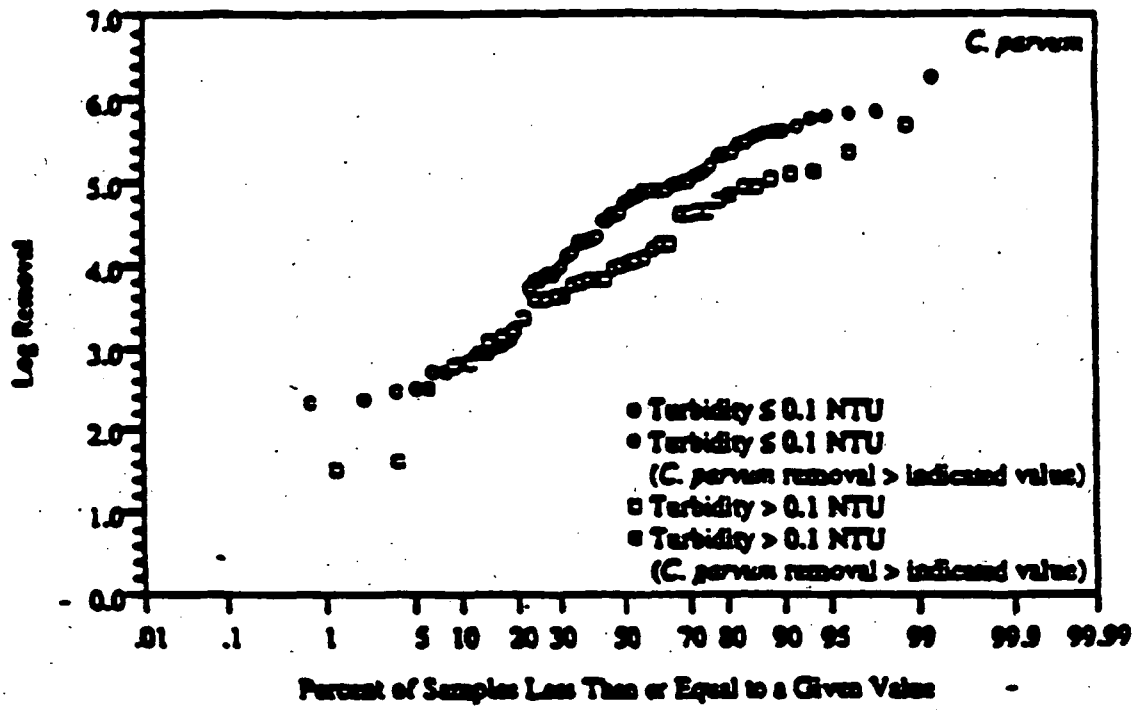


Figure 4-2. Cumulative Probability Distribution of Aggregate Pilot Plant Data for *C. parvum* Removal When Filtered Water Turbidity Was ≤0.1 NTU and >0.1 NTU.

○,□ Oocysts detected
●,■ Oocysts not detected

Source: Patania et al. 1995 cited in EPA 1997.

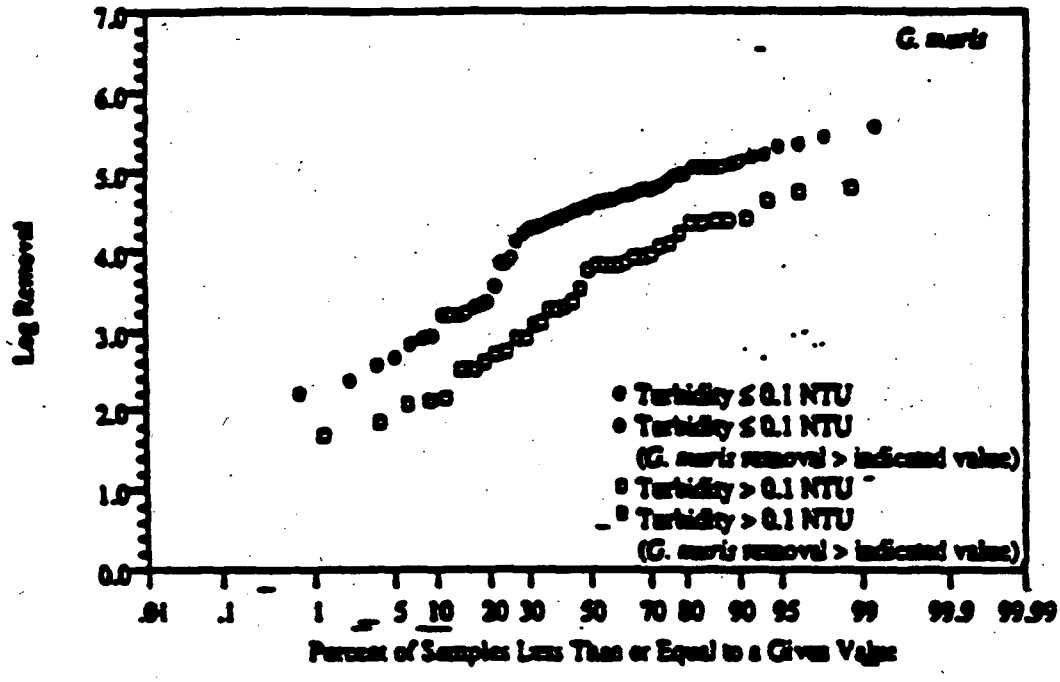


Figure 4-3. Cumulative Probability Distribution of Aggregate Pilot Plant Data for *G. muris* Removal When Filtered Water Turbidity Was ≤0.1 NTU and >0.1 NTU.

○,□ Oocysts detected
●,■ Oocysts not detected

Source: Patania et al. 1995 cited in EPA 1997.

Nieminski and Ongerth (1995) evaluated performance in a pilot plant and in a full-scale plant (not in operation during the time of the study) and considered two treatment modes: direct filtration and conventional treatment. The source water of the full-scale plant typically had turbidities between 2.5 and 11 NTU with a peak level of 28 NTU. The source water of the pilot plant typically had turbidities of 4 NTU with a maximum of 23 NTU. For the pilot plant, achieving filtered water turbidities between 0.1-0.2 NTU, *Cryptosporidium* removals averaged 3.0 logs (range = 1.9 to 4.0 logs) for conventional treatment and 3.0 logs (range = 1.9 to 3.0 logs) for direct filtration, while the respective *Giardia* removals averaged 3.4 logs and 3.3 logs (ranges = 2.2 to 3.9 and 2.2 to 4.0 logs, respectively). For the full-scale plant, achieving similar filtered water turbidities, *Cryptosporidium* removal averaged 2.25 logs for conventional treatment and 2.8 logs for direct filtration, while the respective *Giardia* removals averaged 3.3 logs for conventional treatment and 3.9 logs for direct filtration. The differences in performance noted between direct filtration and conventional treatment in the full-scale plant were attributed to differences in source water quality during the respective filter runs.

Ongerth and Pecoraro (1995) studied the effect of coagulation on protozoa removal from very low turbidity source waters (0.35 to 0.58 NTU). With optimal coagulation, 3-logs removal for both cysts and oocysts was obtained. In one test run, where coagulation was intentionally suboptimal, the removals were only 1.5 logs for *Cryptosporidium* and 1.3 logs for *Giardia*. This emphasized the importance of proper coagulation for cyst removal even though the effluent turbidity was less than 0.5 NTU.

LeChevallier et al. (1991b) evaluated removal efficiencies for *Giardia* and *Cryptosporidium* in 66 surface water treatment plants in 14 States and 1 Canadian province. Most of the utilities achieved between 2 and 2.5 log removals for both *Giardia* and *Cryptosporidium*. When no cysts were detected in the finished water below detection limits, protozoan levels were set at the detection limit for calculating removal efficiencies.

LeChevallier and Norton (1992) evaluated protozoa removal at source water turbidity levels ranging from less than 1 to 120 NTU. Removals of *Giardia* (2.2 to 2.8 logs) and *Cryptosporidium* (2.7 to 3.1 logs) were slightly less than those reported by other researchers, possibly because the full-scale plants were studied, under less ideal conditions than the pilot plants. The participating treatment plants were in varying stages of treatment optimization. Removal achieved a median of 2.5 logs for *Cryptosporidium* and *Giardia*.

In a study conducted in Britain, raw water turbidity ranged from 1 to 30 NTU. *Cryptosporidium* oocyst removal was between 2 and 3 logs (Foundation for Water Research, Britain 1994). Investigators concluded that any measure which reduced filter effluent turbidity should reduce risk from *Cryptosporidium*. The importance in selecting coagulants, dosages, and pH should not be overlooked. Apart from turbidity, indicators of possible reduced efficiency for oocyst removal would be increased color and dissolved metal ion coagulant concentration in the effluent, for these are indications of reduced efficiency of coagulation/flocculation.

Kelley et al. (1995) found protozoa removal in a full-scale conventional plant was between 1.5 and 2 logs. The authors speculated that this low *Cryptosporidium* removal occurred because the coagulation process was not optimized, though the finished water turbidity was less than 0.5 NTU. Also, when oocysts were not detected in the finished water below detection limits, values were assumed as filtered water concentration levels.

West et al. (1994) used pilot-scale direct filtration with anthracite monomedia at filtration rates of 6 and 14 gpm/ft^2. Raw water turbidity was 0.3 to 0.7 NTU. Removal efficiencies for *Cryptosporidium* at both filtration rates were 2 logs during filter ripening (despite turbidity exceeding 0.2 NTU), and 2 to 3 logs for the stable filter run, declining significantly during particle breakthrough. When effluent turbidity was less than 0.1 NTU, removal typically exceeded 2 logs. Log removal of *Cryptosporidium* generally exceeded that for particle removal.

The studies described above indicate that conventional and direct filtration, when operated under appropriate coagulation conditions and achieving a filtered water turbidity level of less than 0.3 NTU, should achieve at least 2 logs of *Cryptosporidium* removal. Removal rates vary widely, up to almost 6 logs, depending upon water matrix conditions, filtered water turbidity levels, and where and when removal efficiencies are measured within the filtration cycle. The highest log pathogen removal rates occurred in those pilot plants and systems which achieved very low finished water turbidities (less than 0.1 NTU).

Other filtration technologies include slow-sand and diatomaceous earth filtration. EPA 1988 listed research studies indicating that a well designed and operated plant using these technologies is capable of removing 3 to 4 logs of *Giardia* and viruses. Schuler and Ghosh (1990) achieved >3 logs removal of *Cryptosporidium* and *Giardia* with diatomaceous earth filtration. Timms et al. (1995) reported 4.5 log removal of *Cryptosporidium* by sand filtration. Removal is less efficient for slow-sand filters at near-freezing temperatures (Fogel et al. 1993).

Membrane processes, such as microfiltration, have achieved greater than 4.8 log removal under bench-scale worst case operating conditions, and 6 to 7 log removal under pilot plant normal operating conditions (Jacangelo et al.1995) (Table 4-6). This is much greater than the log removals observed by other filtration technologies such as slow-sand and diatomaceous earth filtration.

Pre-Treatment Optimization and Filtration Characteristics

Coagulation Effects. Pilot scale studies were conducted to optimize coagulation/filtration processes for the removal of Cryptosporidium oocysts during direct filtration. Either liquid aluminum sulfate (alum) or ferric chloride ($FeCl_3$) was used as the primary coagulant in combination with cationic, anionic, and/or nonionic polymers to arrive at the optimal coagulation conditions for turbidity and particle removal. Each coagulant/polymer combination was evaluated only after stable, consistent filter operation had been demonstrated, typically requiring pilot-plant operation over two or three complete filtration cycles to establish steady-state performance. In the pilot-scale testing, ferric chloride-treated water generally provided slightly greater removal of turbidity, particles, and aerobic spores than alum-treated water, and the filter runs were

shortened to 21 hours from 32 hours for alum-treated water. Coagulation with $FeCl_3$ provided greater *Cryptosporidium* oocyst removal than alum at ambient pH. Oocyst removals of 4.5 $\log_{10}$ were found during filter challenges when $FeCl_3$ was used in conjunction with pre-chlorination and addition of filter aid (polyDADMAC). Similar conditions using alum yielded about 3.7 $\log_{10}$ removal (Yates et al. 1997).

Breakthrough Effects. A 1996 pilot plant study by Hall and Croll assessed aspects of rapid gravity filter operation that can influence the risk of breakthrough into filtered water. They observed quality changes that can occur during a filter run in relation to filtered water particle counts, turbidity, and oocyst concentrations. Initial peaks in particle counts, turbidity, and oocyst concentrations (average 6.3 oocysts per liter) occurred in the first hour of the filter run. Stop and restart after backwashing the filters also produced peaks in particle counts and turbidity, but these were less significant than peaks at the beginning. The initial peaks were a consistent feature of all filter runs monitored at the plant and were attributed to backwashing and filter "ripening." Backwashing of filters will loosen accumulated solids, which may not be removed completely from the bed during the wash and will be released into the filtered water upon restart of the filter. Filter ripening relates to the accumulation of solids within the filter bed that progressively improves the performance for particle removal over the duration of treatment. Their observations demonstrated that higher oocyst concentrations are detected during the first hour of a filter run, consistent with turbidity and particle count data, thus indicating that breakthrough of particles gives a good indication of risk from *Cryptosporidium* (Hall and Croll 1996).

Sedimentation and Dissolved-Air Flotation. The need for proper coagulation and charge neutralization to prepare the oocyst/particle complex for removal by filtration is crucial. A 1995 study by Plummer et al. investigated the effectiveness of dissolved-air flotation (DAF) for the removal of *Cryptosporidium parvum* oocysts, including a comparison with removal levels by conventional treatment with sedimentation. In Table 4-7, comparison of the pre-filtration steps of the conventional treatment process showed only 0 to 0.81 log removal of oocysts by sedimentation and 0.38 to 3.7 log removal by dissolved air flotation processes, depending on coagulant dose (Plummer et al.1995). This combined with results suggesting the buoyancy of oocysts and their low settling velocity (Gregory 1994, Swabby-Cahill et al.1996) demonstrates that pre-filtration processes in treatment are probably not conducting significant oocyst removal and, therefore, filtration is the most important step in removing particulate matter and oocysts.

4.2 DIRECT CONTACT TRANSMISSION

In addition to waterborne transmission, cryptosporidiosis is transmitted by direct contact, including animal-to-human and human-to-human routes. Like waterborne transmission, direct contact transmission occurs by the fecal-oral route. Direct contact transmission of *C. parvum* can occur independently or concurrently with waterborne outbreaks and thus confound the identification of the cause of some epidemics.

This section discusses evidence for direct contact transmission of cryptosporidiosis and, to a lesser extent, giardiasis. The role of animals and humans as reservoirs of *C. parvum* and *Giardia lamblia* is also discussed.

Table 4-7. Data Comparing Sedimentation and Dissolved Air Flotation Removal of *Cryptosporidium*

Raw water oocyst conc. (no./L)	Ferric chloride conc. (mg/L)	Removal by dissolved air flotation (log units)	Removal by sedimentation (log units)
3.5×10^5	2	0.38	0
3.5×10^5	3	2	NR
3.5×10^5	3.5	2.6	0.61
3.5×10^5	4	NR	0.81
3.5×10^5	5	3.7	NR

4.2.1 Animal to Human Transmission

Human infections with *Cryptosporidium* can be derived directly or indirectly from animals, particularly cattle. This type of transmission is commonly referred to as zoonotic transmission (Trees 1997). Infected neonatal calves have been known to be a source of transmission to humans who care for them (Reese et al. 1982, Badenoch et al. 1990). Inhalation of aerosols coughed up by *Cryptosporidium* infected animals caused infection of veterinary attendants (Blagburn and Current 1983, Hojlyng et al. 1987). Table 4-8 summarizes evidence for cross-transmission potential for *Cryptosporidium* from animals to humans.

Evidence exists to implicate direct contact with sheep in human cryptosporidiosis. In an outbreak in North Wales, United Kingdom, cryptosporidiosis in two related cases was associated with exposure to infected bottle-fed orphan lambs (Casemore 1989). Also in the United Kingdom, studies have shown oocysts to be present in the feces of cats (with and without diarrheal symptoms) and wild rodents, although transmission to humans was not reported (Trees 1997). There are no confirmed instances of transmission of *C. parvum* from household pets to humans. However, a study in an Atlanta, Georgia, animal shelter found that 10 percent of puppies examined in the shelter were infected with *C. parvum* (Jafri et al. 1993).

Human infections from *Giardia* cysts derived from beavers and mule deer have been reported (Davies and Hibler 1979). Likewise, cross-species transmission from humans to other animals, including dogs, beavers, muskrats, laboratory rats, gerbils, raccoons, bighorn sheep, suckling mice, and guinea pigs, has been demonstrated (Hewlett et al. 1982, Craft 1982, Davies and Hibler 1979, Hill et al. 1983, Sheffield and Bjorvatn 1977, Belosevic et al. 1983). Although there have been some concerns about experimental design and confirmation of use of *Giardia*-free animals, results indicate definite cross-species transmission of *Giardia*; furthermore, the experimental infections can be established by direct feeding with doses of either cysts or

Table 4-8. Cross-Transmission Potential between Animals and Humans for *Cryptosporidium*

Animal	Prevalence of infection in animals	Evidence for transmission	
		Animals to humans	Humans to animals
Dogs	1.4–45%	No	Yes
Cats	1.3–87%	Yes[a]	Yes
Cattle[b]	17–76%	Yes[a]	Yes
Dairy calves	50%	Yes	Yes
Sheep	78%	No	No (not tested)
Goats	—[c]	No	No (not tested)
Deer[d]	92%		
Pigs	5.3%	Yes[a]	Yes
Horses	16%	No	No (not tested)
Raccoons	13%	No	No (not tested)
Mice	30%	No	Yes
Rats[e]	—[c]	No	Yes
Rabbits	8%	No	No (not tested)
Chickens[f]	5.9–27%	No	No
Ducks[g]	88%	No	No

Sources: Dubey et al. 1990, Juranek 1995, Lindsay and Blagburn 1990, Wang 1991, as cited in Rose 1997.
[a] Epidemiological evidence, over five studies documenting transmission from cattle.
[b] Over 15 studies, antibody prevalence indicating infection is 50% in calves and >90% on farms.
[c] Infection prevalence is unknown.
[d] High prevalence in farmed deer, unknown in the wild but has been documented in the roe deer, fallow deer, sika deer, mule deer, Eld's deer, axis deer, and barasingha deer. All were found in diarrheic neonatal deer.
[e] Also found in guinea pigs and hamsters.
[f] Also found in turkeys, ducks, pheasants, quail, geese, and miscellaneous birds (love birds); quail isolate is able to infect mammals.
[g] At duck farms.

cultured trophozoites (EPA 1996a). It is possible that with the development of more effective methods for classifying *Giardia lamblia* isolates of various host origins (Baruch et al. 1996), the epidemiology of zoonotic transmission of giardiasis can be more accurately determined.

4.2.2 Human to Human Transmission

Transmission of *Cryptosporidium* infection between persons may occur in families, daycare centers, hospitals, and urban environments where high population densities exist (Baxby et al. 1983, Brown et al. 1989, Ribeiro and Palmer 1986, Heijbel et al. 1987, Melo Cristino et al. 1988, Fayer et al. 1990, Casemore et al. 1997). Juranek (1995) explains that children in diapers are at especially high risk for direct transmission of cryptosporidiosis because of intimate play or careless diaper changing practices. Attack rates of over 60 percent in urban day care centers have been reported (Fayer and Ungar 1986). Both sporadic and daycare center outbreaks are often associated with confirmed secondary cases among family members and other people who have had recent contact with infected individuals. Asymptomatic family members living with confirmed cases are sometimes found to excrete oocysts in small numbers.

Hospital cross infection with *C. parvum*, such as from patient to staff, has been documented and is further evidence of human-to-human transmission (Tzipori et al. 1983, Crawford and Vermund 1988, Casemore et al. 1997). *Cryptosporidium* has been found in sputum and in vomit (Tzipori et al. 1983). Nosocomial cryptosporidiosis infections have been reported in both hospital staff and patients (Juranek 1995). Fecal-oral exposure during sexual contact has been implicated as a transmission (exposure) route for direct-contact transmission in homosexual males with acquired immunodeficiency syndrome (AIDS). Respiratory cryptosporidiosis has been reported, sometimes in the absence of diarrhea (Mifsud et al. 1994, Clavel et al. 1996, Dupont et al. 1996).

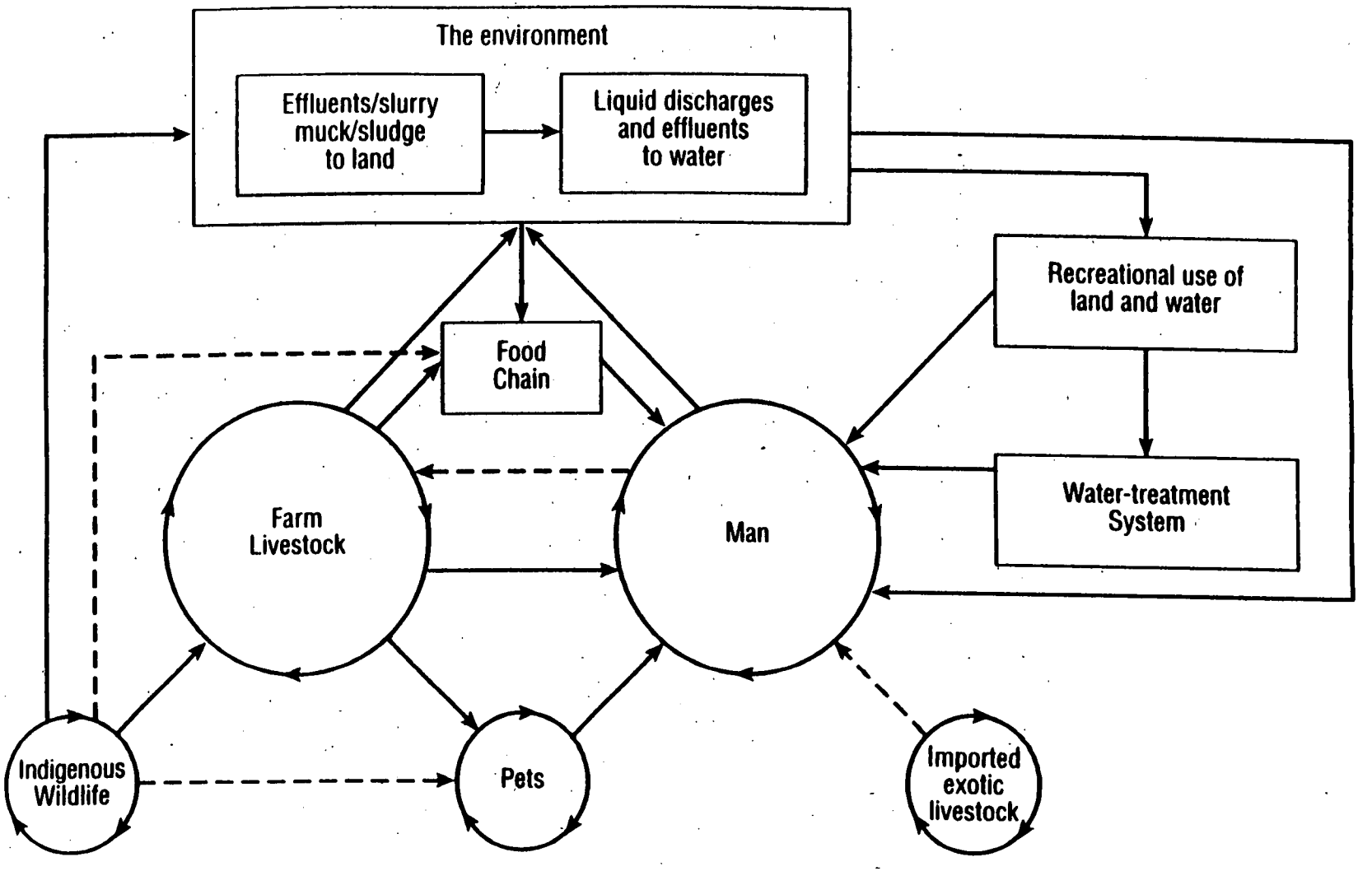
Because both *Cryptosporidium* and *Giardia* are distributed worldwide, data from daycare centers in Salamanca, Spain, are applicable to the question of human transmission. Rodriguez-Hernandez et al. (1996) studied 170 children younger than 4 years old who regularly attended daycare centers. *Giardia* was the most frequently identified parasite (25.3 percent, or 43 children); 10 percent of children (17) were parasitized by *Cryptosporidium*. Cryptosporidiosis was more frequent in winter than in other seasons, and giardiasis was more frequent in autumn.

The various reservoirs for human infection include humans, livestock, pets, wildlife, food, surface and recreational waters (e.g., swimming pool), and wastewater (Casemore 1990). Domestic animal reservoirs are significant sources of oocysts in unprotected watersheds, where the parasites may remain viable for extended periods. For example, *C. parvum* oocysts may be shed in numbers of up to 10^5 to 10^7 per gram in calf feces (Ongerth and Stibbs 1987). Crabb (unpublished, cited in Fricker and Crabb 1998) observed that a newborn calf shedding oocysts for 2 weeks at a concentration of 10^5 to 10^7 per ml can produce 5×10^{10} total oocysts. The USDA (1993) found animals excreting *Cryptosporidium* oocysts in 90 percent of farms surveyed in 1991-1992 in the northeast, midwest, and western states (USDA 1993, cited in Fricker and Crabb 1998). Common reservoirs of *Cryptosporidium* are illustrated in Figure 4-4.

4.3 FOODBORNE TRANSMISSION

Although historically difficult to identify positively in food samples, foodborne transmission of *Cryptosporidium* was suspected in the United Kingdom as the cause of gastroenteritis following the occasional (versus regular) consumption of raw foods such as fresh sausage, raw milk, and offal (the viscera and trimmings of butchered animals) (Casemore et al. 1997). Regular consumption of raw vegetables contaminated with low levels of oocysts was thought to confer some level of immunity to the disease (unpublished United Kingdom outbreak reports of the Public Health Laboratory Service, Communicable Disease Surveillance Centre, cited in Casemore et al. 1997). Molecular probes have improved the ability to document food contamination by *Cryptosporidium* (Bankes 1994).

The incidence of foodborne outbreaks of cryptosporidiosis in the U.S. has been reported sporadically (Smith 1993). Millard et al. (1994) first documented foodborne transmission of *Cryptosporidium* as the cause of a 1993 U.S. outbreak of cryptosporidiosis at an agricultural fair in Maine. There were 160 primary cases and 53 secondary cases associated with the consumption of unpasteurized apple cider during that outbreak



Source: Casemore et al. 1997

Figure 4-4. Reservoirs of Infection and Routes of Transmission of *Cryptosporidium parvum*. Solid lines represent recognized routes of transmission: broken lines represent probable routes of transmission. (Modified from Casemore, D.P., *Epidemiol. Infect.*, 104, 1, 1990.)

(Millard et al. 1994). In all, approximately 26 percent of the fair attendees were affected. Oocysts were recovered from the cider, the cider press, and from a stool specimen from a calf on the farm where the apples had been harvested (Millard et al. 1994).

In September 1995, CDC investigated an outbreak of foodborne cryptosporidiosis in Blue Earth County, Minnesota. The outbreak was associated with the ingestion of chicken salad that had been prepared for a social event by the operator of a licensed day care home. Fifty persons attended the event, and of 26 of these persons who completed phone interviews with CDC, 15 (58 percent) reported onset of watery diarrhea within 14 days after attending the event (Besser-Wiek et al. 1996). There were no other reports of cryptosporidiosis in the community at the time of the outbreak, and water consumption was not associated with the illness. Although the preparer of the contaminated food denied having recent diarrhea, she acknowledged that she had changed infant diapers before preparing the salad (Besser-Wiek et al. 1996).

4.4 SUMMARY

Contaminated sewage and animal feces serve as sources of oocyst and cyst contamination of drinking water when effluents and agricultural runoff are transported to a watershed above the intake of a water treatment system or adjacent to a well that induces surface water infiltration. Although generally recognized as ubiquitous in the environment, oocyst concentrations in raw water have been shown to vary with the level of human activities in a watershed including recreational use, discharge of sewage, and discharges from agricultural areas. The oocysts, which are resistant to chlorination and other chemical disinfectants, can be removed by filtration. The number of logs of oocyst removal observed in pilot and full-scale filtration systems varies from approximately 1.5 to 7 $\log_{10}$, but filtration beds have been shown to vary in removal efficiency, especially after backwashing. Conventional or direct filtration in a well designed and operated plant can remove 3 to 4 logs of *Giardia*.

Transmission of cryptosporidiosis and giardiasis is by the fecal-oral route, and both waterborne and direct contact transmission routes have been well documented. *C. parvum* and *G. lamblia* lack species specificity and can be transmitted zoonotically (from animals to humans) or from human to human (e.g., among toddlers in a daycare setting). Because the oocysts and cysts are shed in the feces of infected animals and humans at rates up to 10^{10} oocysts per day, both animals and humans can act as reservoirs of the disease; challenge studies have shown that both symptomatic and asymptomatic carriers shed oocysts and cysts.

5. OCCURRENCE OF PATHOGENS IN DRINKING WATER

Evidence for the occurrence of *Cryptosporidium parvum* and *Giardia lamblia* in drinking water supplies is available from epidemiological surveillance reports and from a limited number of surveys and monitoring studies. Section 5.1 presents an overview of recent surveillance data documenting incidence of waterborne disease in the United States and its territories. Section 5.2 discusses outbreaks of cryptosporidiosis in greater detail, including *Cryptosporidium* recovery in raw and/or finished water from several case studies. Limited data on *Cryptosporidium* detected in water in association with cryptosporidiosis outbreaks is presented. This section also presents survey and monitoring data that document the occurrence of *Cryptosporidium* in raw and finished water supplies, including both surface water and groundwater under the direct influence of surface water. Caveats on interpreting data obtained with currently available methods are discussed. A brief discussion of outbreak and monitoring data for *Giardia* in surface water and groundwater are discussed in Section 5.3.

5.1 WATERBORNE DISEASE OUTBREAKS IN THE UNITED STATES

This section presents an overview of waterborne disease outbreaks caused by infectious or unknown agents. The rate at which new cases of a waterborne disease such as cryptosporidiosis occur in a population is referred to as the "incidence" of that disease in the population; "prevalence" measures both new and old cases of the disease in the population (Craun et al. 1996). Calculation of the incidence rate takes into account the number of cases of disease per person-year (or other person-time unit) of exposure. Other outbreak-specific information including geographic clustering of the cases, estimated rate of water consumption (for waterborne transmission), correlation of primary cases with transmission sources ("vehicle"- specific factors), and rates of secondary transmission are also examined during investigation of an outbreak or a group of outbreaks (Craun et al. 1996). When these data are incomplete or unreliable, causal associations and etiologies (i.e., the origins) of outbreaks may be difficult to establish.

Figure 5-1 illustrates the number of waterborne disease outbreaks in the United States in water intended for drinking each year between 1980 and 1994 (adapted from Kramer et al. 1996). To be considered a waterborne outbreak, incidents of acute gastroenteritis must affect at least two persons and be associated epidemiologically with ingestion of water (Craun and Calderon 1996). The figure shows that the number of reported outbreaks in recent years is lower than the number of outbreaks that were reported during the 1980s.

The CDC and EPA have maintained a collaborative surveillance program for collection and periodic reporting of data on waterborne disease outbreaks since 1971. The CDC database and biennial CDC-EPA surveillance summaries cited in this document include data reported voluntarily by the states on the incidence and prevalence of waterborne illnesses. However, epidemiologists believe the occurrence of outbreaks of waterborne gastrointestinal infections including cryptosporidiosis may be much greater than suggested by reported surveillance data (Craun and Calderon 1996). Cryptosporidiosis is self-limiting in healthy adults;

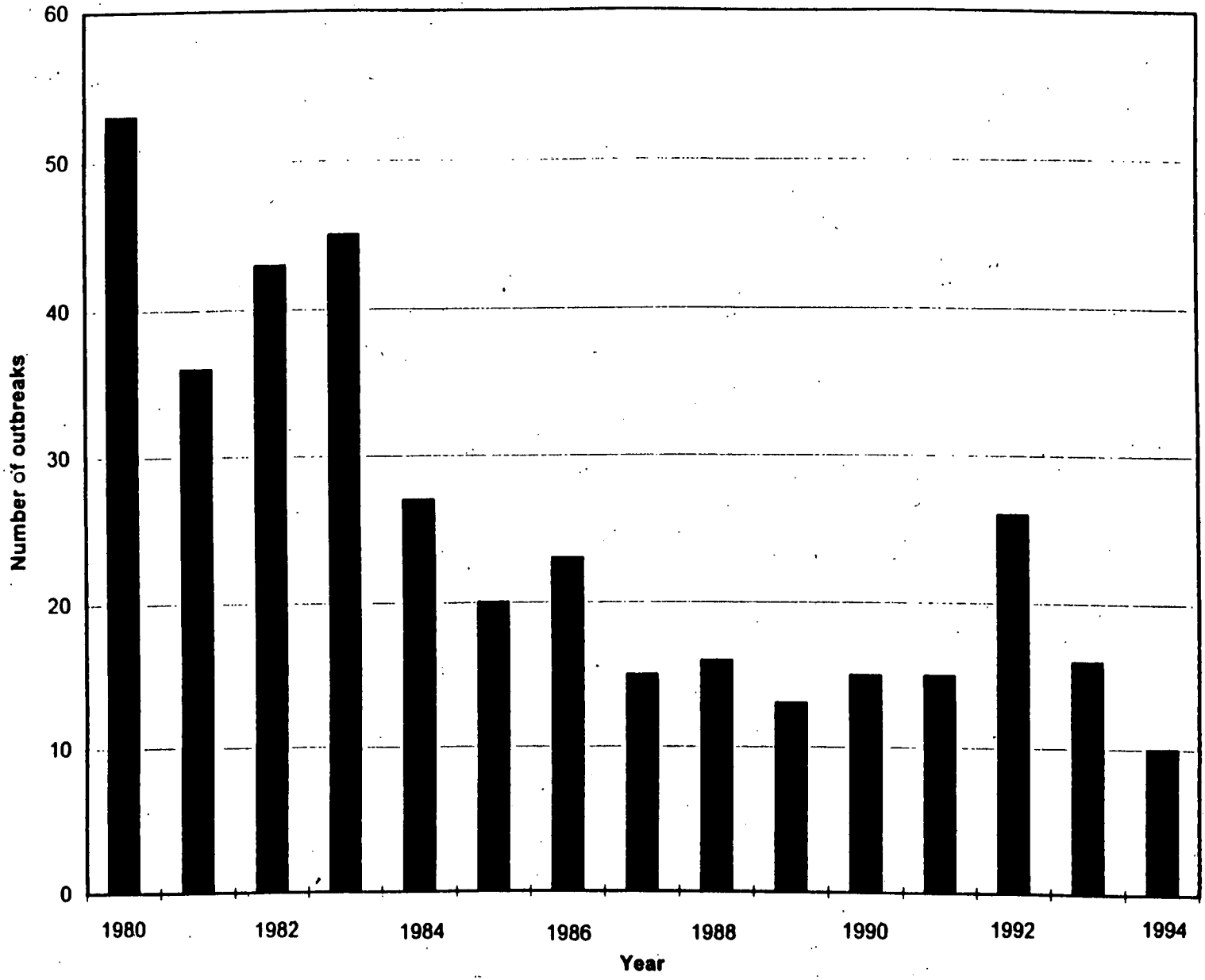


Figure 5-1. Waterborne Disease Outbreaks 1980-1994.

Adapted from Figure 4 (J. AWWA 1996, vol. 88, no. 3), as corrected for several missing outbreaks by G.F. Craun. Source: Kramer et al. 1996. [98-089XLS]

therefore, in the absence of an outbreak, individual cases may not be treated clinically or reported. Furthermore, *Cryptosporidium* is not analyzed by routine diagnostic tests for gastroenteritis and, therefore, tends to be under-reported in the general population (Juranek 1995, Craun 1996).

Outbreaks of cryptosporidiosois and giardiasis have been documented in community, non-community, and private water systems in the U.S. (Moore et al. 1993, Kramer et al. 1996, Craun 1996). CWSs provide water to at least 15 service connections used by year-round residents or regularly serve at least 25 year-round residents (EPA 1998b). Non-community water systems include many NTNC water systems that are not CWSs and regularly serve at least 25 of the same persons over six months per year; common types of NTNC water systems are those serving schools, day care centers, factories, restaurants, nursing homes, and hospitals (EPA 1998b). Private wells have also become contaminated (Kramer et al. 1996). Craun (1996) estimated that usually one percent of populations served by CWSs and 2 percent of populations served by noncommunity water systems must become reportably ill before an outbreak is recognized by public health agencies.

At present, a number of factors contribute to the underreporting of waterborne illnesses including cryptosporidiosis and giardiasis in communities in the U.S. Among these factors are: limitations of federal, state, and local surveillance systems, difficulties in assessing the threat to drinking water when oocysts are detected in finished water supplies (related to uncertainties the monitoring method), and underreporting of endemic or asymptomatic infections in communities.

The current surveillance system for waterborne illness lends itself to underreporting of individual cases of gastrointestinal illness, including those caused by protozoa. Figure 5-2 summarizes the sequence of events that must occur before an individual gastrointestinal infection can be reported under typical state surveillance systems (Frost et al.1996). Limitations of the current state-federal surveillance system for waterborne disease include the following (Frost et al. 1996):

- most state surveillance systems are passive (more cases could be identified by active surveillance systems);
- primary reporting responsibility in most systems resides in the individual health care providers and diagnostic laboratories;
- enforcement of reporting requirements by the states is minimal; and
- disease reporting is incomplete in both passive and active surveillance systems, and the degree of underreporting of cases varies among states and among areas within states.

Also, because viability and infectivity of oocysts and cysts detected in finished water samples cannot be determined by the analytical method used for monitoring, water purveyors that detect *Cryptosporidium* or *Giardia* in finished water supplies cannot accurately assess the risk to consumers of such findings (Frost et al. 1997). Because of these reasons, the CDC recommends that the sole detection of *Cryptosporidium* in

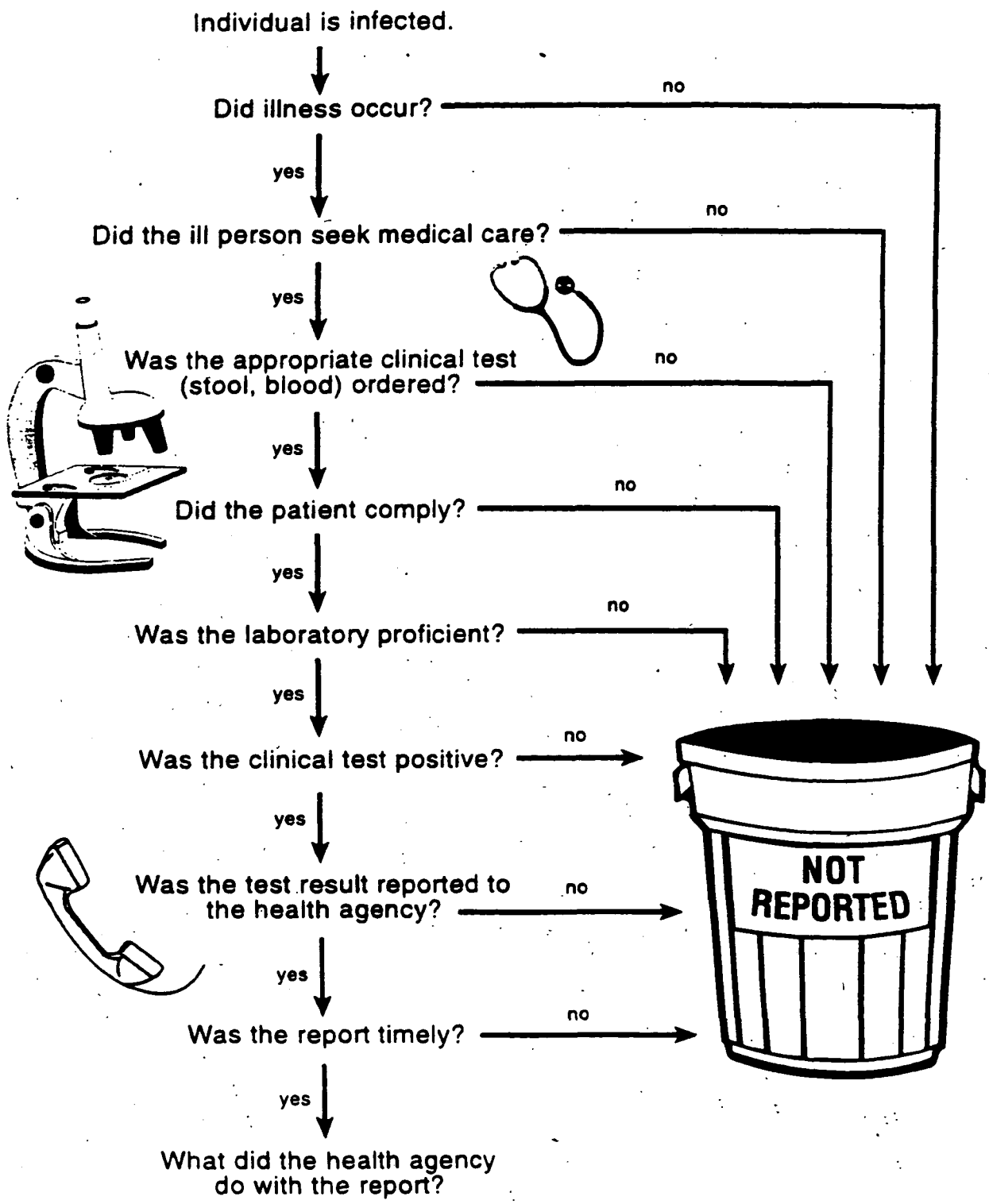


Figure 5-2. Sequence of Events Before an Individual Infection Can Be Reported.
Source: Frost et al. 1996.

finished water is not enough to warrant any boil-water advisory in a community (CDC 1995a). If the public is not be alerted to the occurrence of *Cryptosporidium* in the water supply, underreporting of gastrointestinal illness may occur, especially if the patients do not seek clinical treatment.

Some epidemiologists believe that cryptosporidiosis and other waterborne diseases are underreported because not all infections by enteric pathogens result in symptomatic illness (Craun 1996, Frost et al. 1997). Evidence exists that short-term immunity or protection from cryptosporidiosis occurs in some immunocompetent persons after an initial infection (Frost et al.1997). Other persons may be infected but have mild symptoms or remain asymptomatic (Craun 1996). Such "endemic disease", defined by CDC as a persistent low to moderate background level of disease occurrence in a population (Craun et al.1996) often goes undetected by routine disease surveillance programs.

When prior exposure or chronic contamination of the water by low levels of oocysts confers short-term immunity to immunocompetent residents in a community (Okhuysen et al. 1998), most cases of symptomatic illness in that community will then occur in newly exposed individuals, such as young children, visitors, and new residents (Frost et al. 1997). The authors caution that sensitive populations may be at risk of severe illness from either epidemic or endemic infection by *Cryptosporidium* (Frost et al. 1997).

During an outbreak, cases of cryptosporidiosis may be confirmed by clinical analysis of fecal specimens or by serological testing. Even when tests for *Cryptosporidium* in fecal specimens are ordered, the organism may be missed (Casemore et al. 1997). For example, false negatives in specimens obtained from children tested early in their illness during the 1993 Milwaukee, WI cryptosporidiosis outbreak suggested either a delayed or intermittent excretion of oocysts by those patients, or a failure of the tests to detect the oocysts (Cicerello et al 1997, cited in Casemore 1997). A recent study of factors affecting *Cryptosporidium* testing in Connecticut found testing practice to be inadequate to allow detection of outbreaks or assess their impact (Roberts et al. 1995, cited in Casemore et al. 1997). Evidence of exposure to *Cryptosporidium* or other pathogens also may be obtained by serological testing. A recent serological study of Peace Corps workers before they went overseas indicated that almost 30 percent of the workers had increased antibody levels specific to *Cryptosporidium* before they left the U.S., suggesting that they had already been exposed to the parasite (Ungar et al. 1989). Frost et al. (1997) speculate that routine serological surveys of a community, conducted as part of a systematic surveillance program, will identify endemic as well as epidemic cryptosporidiosis, and help to determine the success of water treatment facilities in preventing protozoal infection.

Tables 5-1 through 5-6 summarize information from the two most recently published CDC-EPA surveillance summaries, Moore et al. (1993) and Kramer et al. (1996) as well as unpublished data for 1995-1996 (Craun 1998). The tables list the annual outbreaks from 1991 through 1996, including the state in which the outbreak occurred, the month the outbreak occurred, the principal etiologic agent (if known), numbers of cases of illness, type of drinking water system (i.e., community or non-community), system deficiencies if determined, type of source water, and community setting. Tables 5-7 through 5-11 present summary statistics for the annual outbreaks, including etiologic agents, type of system deficiency, and type of water system.

Table 5-1. Outbreaks Associated with Water Intended for Drinking: United States, 1991[a]

State[b]	Month	Class[c]	Etiologic agent[d]	No. cases	Type of system[e]	Deficiency[f]	Source	Setting
CA	July	I	*Giardia*	15	NC	4	Spring	Recreation area
IL	May	II	AGI	386	NC	5	Well	School
MI	June	I	AGI	1,320	NC	2	Well	Campground
MI	August	I	AGI	33	NC	2	Well	Resort
MN	June	I	AGI	30	NC	2	Well	Campground
MN	July	I	AGI	30	NC	4	Well	Resort
MN	August	I	AGI	17	NC	2	Well	Restaurant
NM	August	I	AGI	38	NC	2	Well	Camp
PA	June	I	AGI	170	NC	3	Well	Picnic area
PA	July	I	AGI	8	NC	3	Well	Restaurant
PA	Sept	III	*Giardia*	13	NC	3	Well	Park
PA	August	I	*Cryptosporidium*	551	NC	3	Well	Picnic area
PA	June	I	AGI	300	NC	3	Well	Camp
PR	August	I	AGI	202	Com	4	River	Penitentiary
PR	August	I	AGI	9,847	Com	3	River	Community

[a] Does not include outbreaks caused by chemical contaminants.

[b] Includes territories.

[c] Class I = adequate epidemiologic and water quality data; Class II = adequate epidemiologic data only and water quality data not provided or inadequate; Class III = limited epidemiologic data provided and adequate water quality data provided.

[d] AGI = acute gastrointestinal illness of unknown etiology.

[e] NC = noncommunity; Com = community; Ind = individual.

[f] Definitions of deficiencies: (1) Untreated surface water; (2) untreated groundwater; (3) treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, and inadequate or no filtration); (4) distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, and contamination of a storage facility); and (5) unknown or miscellaneous deficiency (e.g., contaminated bottled water).

Source: Moore et al. 1993.

Table 5-2. Outbreaks Associated with Water Intended for Drinking: United States, 1992[a]

State[b]	Month	Class[c]	Etiologic agent[d]	No. cases	Type of system[e]	Deficiency[f]	Source	Setting
ID[1]	Mar	III	*Giardia*	15	Com	2	Well	Trailer park
MN[1]	Feb	I	AGI	250	NC	3	Lake	Restaurant
NV[1]	Mar	I	*Giardia*	80	Com	3	Lake	Community
NY[1]	Apr	III	AGI	107	NC	4	Well	Restaurant
NC[1]	Jan	I	AGI	200	NC	2	Well	Restaurant
OH[1]	Jun	III	AGI	129	NC	4	Well	Campground
OR[1]	Feb	I	*Cryptosporidium*		Com	3	Spring	Community
OR[1]	May	I	*Cryptosporidium*		Com	3	River	Community
PA[1]	Mar	III	AGI	5	NC	3	Well	Restaurant
PA[1]	May	II	AGI	28	Com	5	River	Park
PA[1]	Jun	III	AGI	38	Ind	2	Well	Private home
PA[1]	Jun	III	AGI	42	NC	3	Well	Camp
PA[1]	May	I	AGI	50	NC	3	Well	Camp
PA[1]	May	III	AGI	57	NC	3	Well	Camp
PA[1]	Aug	I	AGI	80	NC	3	Well	Camp
WA[1]	Jun	I	*Hepatitis A*	10	Ind	2	Well	Private home
WY[1]	Jul	I	*Shigella sonnei*	150	NC	2	Well	Park
MN[2]	May	I	AGI	70	NC	5	Well	Hotel
MO[2]	Jan	III	*Shigella sonnei*	5	I	4	Cistern	Private home
MO[2]	Apr	III	Hepatitis A virus	46	NC	2	Well	School/church
MO[2]	Jul	I	*Shigella sonnei*	11	NC	4	Well	Campground
PA[2]	May	I	AGI	50	NC	3	Well	Camp

[a] Does not include outbreaks caused by chemical contaminants.

[b] Includes territories.

[c] Class I = adequate epidemiologic and water quality data; Class II = adequate epidemiologic data only and water quality data not provided or inadequate; Class III = limited epidemiologic data provided and adequate water quality data provided.

[d] AGI = acute gastrointestinal illness of unknown etiology.

[e] NC = noncommunity; Com = community; Ind = individual.

[f] Definitions of deficiencies: (1) Untreated surface water; (2) untreated groundwater; (3) treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, and inadequate or no filtration); (4) distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, and contamination of a storage facility); and (5) unknown or miscellaneous deficiency (e.g., contaminated bottled water).

Sources: [1]Moore et al. 1993.
[2]Kramer et al. 1996.

Table 5-3. Outbreaks Associated with Water Intended for Drinking: United States, 1993[a]

State[b]	Month	Class[c]	Etiologic agent[d]	No. cases	Type of System[e]	Deficiency[f]	Source	Setting
MN	Aug	II	*Cryptosporidium parvum*	27	NC	5	Lake	Resort
MN	Nov	I	*Campylobacter jejuni*	32	NC	4	Well	Resort
MO	Dec	I	*Salmonella* serotype[g]	625	Com	4	Well	Community
NV	Dec	II	*C. parvum*[h]	103	Com	5	Lake	Community
NY	Jun	III	*Campylobacter jejuni*	172	Com	5	Well	Subdivision
PA	Jan	III	*Giardia lamblia*	20	Com	3	Well	Trailer park
PA	Mar	I	AGI	65	NC	3	Well	Ski resort
SD	Sept	III	*G. lamblia*	7	Com	2	Well	Subdivision
SD	Sept	I	AGI	40	NC	2	Well	Resort
WA	Apr	III	*C. parvum*	7	Ind	2	Well	Private home
WI	Mar	I	*C. parvum*[i]	403,000	Com	3	Lake	Community

[a] Does not include outbreaks caused by chemical contaminants.

[b] Includes territories.

[c] Class I = adequate epidemiologic and water quality data; Class II = adequate epidemiologic data only and water quality data not provided or inadequate; Class III = limited epidemiologic data provided and adequate water quality data provided.

[d] AGI = acute gastrointestinal illness of unknown etiology.

[e] NC = noncommunity; Com = community; Ind = individual.

[f] Definitions of deficiencies: (1) Untreated surface water; (2) untreated groundwater; (3) treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, and inadequate or no filtration); (4) distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, and contamination of a storage facility); and (5) unknown or miscellaneous deficiency (e.g., contaminated bottled water).

[g] Seven deaths associated with outbreak (Angulo et al. 1997).

[h] Twenty deaths associated with outbreak (Goldstein et al. 1996b).

[i] Fifty deaths associated with outbreak (Wisconsin DHSS 1996).

Source: Kramer et al. 1996.

Table 5-4. Outbreaks Associated with Water Intended for Drinking: United States, 1994[a]

State[b]	Month	Class[c]	Etiologic agent[d]	No. cases	Type of system[e]	Deficiency[f]	Source	Setting
ID	Jun	III	*Shigella flexneri*	33	Ind	2	Well	Private homes
IN	Mar	II	AGI	118	NC	2	Well	Restaurant
ME	Aug	III	AGI	72	NC	2	Well	Camp
MN	Jun	I	*Campylobacter jejuni*	19	NC	2	Well	Park
NH	May	III	*Giardia lamblia*	18	Com	3	Reservoir	Community
NH	May	III	*Giardia lamblia*	36	Com	3	Lake	Community
NY	Jun	III	*Shigella sonnei*	230	NC	2	Well	Camp
Saipan	Jun	III	Non-O1 *Vibrio cholerae*	11	Com	5	Wells	Bottled water
PA	Sept	I	AGI	200	NC	3	Well	Resort
TN	Mar	I	*Giardia lamblia*	304	Com	4	Reservoir	Correctional facility
WA	Aug	I	*Cryptosporidium parvum*	134	Com	2	Well	Community

[a] Does not includes outbreaks caused by chemical contaminants.

[b] Includes territories.

[c] Class I = adequate epidemiologic and water quality data; Class II = adequate epidemiologic data only and water quality data not provided or inadequate; Class III = limited epidemiologic data provided and adequate water quality data provided.

[d] AGI = acute gastrointestinal illness of unknown etiology.

[e] NC = noncommunity; Com = community; Ind = individual.

[f] Definitions of deficiencies: (1) Untreated surface water; (2) untreated groundwater; (3) treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, and inadequate or no filtration); (4) distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, and contamination of a storage facility); and (5) unknown or miscellaneous deficiency (e.g., contaminated bottled water).

Source: Kramer et al. 1996.

Table 5-5. Outbreaks Associated with Water Intended for Drinking: United States, 1995[a]

State[b]	Month	Class[c]	Etiologic agent[d]	No. cases	Type of system[e]	Deficiency[f]	Source	Setting
AK	August	II	*Giardia*	10	Ind	1	Surface	Unknown
ID	August	I	*S. sonnei*	83	NC	2	Well	Resort
ID	Sept.	I	AGI	10	Com	3	Well	Community
MN	July	I	*E. coli 0157:H7*	33	NC	2	Spring	Camp
MT	August	II	AGI	450	NC	2	Well	Campground
NY	Dec.	I	*Giardia*	1449	Com	3	Lake	Community
OK	Oct.	II	*S. sonnei*	10	NC	5	Well	Convenience Store
PA	August	I	AGI	19	NC	2	Well	Inn
SD	June	I	AGI	48	NC	2	Well	Camp
WI	August	III	Rotavirus	26	NC	3	Well	Restaurant
WI	Sept.	I	SRSV	148	Com	4	Lake	School

[a] Does not include outbreaks caused by chemical contaminants.

[b] Includes territories.

[c] Class I = adequate epidemiologic and water quality data; Class II = adequate epidemiologic data only and water quality data not provided or inadequate; Class III = limited epidemiologic data provided and adequate water quality data provided.

[d] AGI = acute gastrointestinal illness of unknown etiology; SRSV = small round structured virus.

[e] NC = noncommunity; Com = community; Ind = individual.

[f] Definitions of deficiencies: (1) Untreated surface water; (2) untreated groundwater; (3) treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, and inadequate or no filtration); (4) distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, and contamination of a storage facility); and (5) unknown or miscellaneous deficiency.

Source: Craun 1998.

Table 5-6. Outbreaks Associated with Water Intended for Drinking: United States, 1996[a]

State[b]	Month	Class[c]	Etiologic agent[d]	No. cases	Type of system[e]	Deficiency[f]	Source	Setting
CA	Sept.	I	AGI	8	Ind	5	Sewage plant	Hikers/use of non-potable water tap
ID	July	III	AGI	94	NC	3	Well	Camp
NY	June	I	*Plesiomonas shigelloides*	60	NC	3	Spring	Catering facility
WI	June	III	AGI	21	NC	4	Well	Restaurant

[a] Does not include outbreaks caused by chemical contaminants.

[b] Includes territories.

[c] Class I = adequate epidemiologic and water quality data; Class II = adequate epidemiologic data only and water quality data not provided or inadequate; Class III = limited epidemiologic data provided and adequate water quality data provided.

[d] AGI = acute gastrointestinal illness of unknown etiology.

[e] NC = noncommunity; Com = community; Ind = individual.

[f] Definitions of deficiencies: (1) Untreated surface water; (2) untreated groundwater; (3) treatment deficiency (e.g., temporary interruption of disinfection, chronically inadequate disinfection, and inadequate or no filtration); (4) distribution system deficiency (e.g., cross-connection, contamination of water mains during construction or repair, and contamination of a storage facility); and (5) unknown or miscellaneous deficiency.

Source: Craun 1998.

Table 5-7. Outbreaks Associated with Water Intended for Drinking, by Etiologic Agent and Type of Water System: United States, 1991–1992[a]

	Type of water system						Total	
	Community		Noncommunity		Individual			
Agent	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases
AGI	3	10,077	21	3,372	1	38	25	13,487
Giardia	2	95	2	28	0	0	4	123
Cryptosporidium	2	3,000	1	551	0	0	3	3,551
Hepatitis A	0	0	1	46	1	10	2	56
Shigella sonnei	0	0	2	161	1	5	3	166
Total	7	13,172	27	4,158	3	53	37	17,383

AGI = Acute gastrointestinal illness of unknown etiology.
Sources: Moore et al. 1993; Kramer et al. 1996.
[a] Excludes outbreaks due to chemical contamination.

Table 5-8. Outbreaks Associated with Water Intended for Drinking, by Type of Deficiency and Type of Water System: United States, 1991–1992[a]

Type of deficiency	Community		Noncommunity		Individual		Total	
	No.	%	No.	%	No.	%	No.	%
1 Untreated surface water	0	0	0	0	0	0	0	0
2 Untreated groundwater	1	13	8	30	1	33	10	27
3 Treatment deficiency	4	57	12	44	1	33	17	46
4 Distribution system deficiency	1	14	5	19	1	33	7	19
5 Unknown	1	14	2	7	0	0	3	8
Total	7	100	27	100	3	100	37	100

Sources: Moore et al. 1993; Kramer et al. 1996.

[a] Does not include outbreaks due to chemical contamination.

Table 5-9. Outbreaks Associated with Water Intended for Drinking, by Etiologic Agent and Type of Water System: United States, 1993–1994[a]

	Type of water system						Total	
	Community		Noncommunity		Individual			
Agent	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases
Cryptosporidium parvum	3	403,237	1	27	1	7	5	403,271
AGI	0	0	5	495	0	0	5	495
Giardia lamblia	5	385	0	0	0	0	5	385
Campylobacter jejuni	1	172	2	51	0	0	3	223
Salmonella typhimurium	1	625	0	0	0	0	1	625
Shigella sonnei	0	0	1	230	0	0	1	230
Shigella flexneri	0	0	0	0	1	33	1	33
Non-0-1 *Vibrio cholerae*	1	11	0	0	0	0	1	11
Total	11	404,430	9	803	2	40	22	405,273

Source: Kramer et al. 1996.
[a] Does not include outbreaks attributed to chemical contamination.

Table 5-10. Outbreaks Associated with Water Intended for Drinking, by Type of Deficiency and Type of Water System: United States, 1993–1994[a]

Type of deficiency	Community		Noncommunity		Individual		Total	
	No.	%	No.	%	No.	%	No.	%
1 Untreated surface water	0	0	0	0	0	0	0	0
2 Untreated groundwater	2	18	5	56	2	100	9	41
3 Treatment	4	36	2	22	0	0	6	27
4 Distribution system	2	18	1	11	0	0	3	14
5 Unknown	3	27	1	11	0	0	4	18
Total	11	100	9	100	2	100	22	100

Source: Kramer et al. 1996.
[a] Does not include outbreaks attributed to chemical contamination.
[b] Percentages may not add to 100% because of rounding.

Table 5-11. Outbreaks Associated with Water Intended for Drinking, by Etiologic Agent and Type of Water System: United States, 1995–1996[a]

	Type of water system						Total	
	Community		Noncommunity		Individual			
Agent	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases
AGI	1	18	5	632	1	8	7	658
Shigella sonnei	0	0	2	93	0	0	2	93
Giardia	1	1449	0	0	1	10	2	1459
SRSV	1	148	0	0	0	0	1	148
Plesiomonas shigelloides	0	0	1	60	0	0	1	60
Escherichia coli 0157:H7	0	0	1	33	0	0	1	33
Rotavirus	0	0	1	26	0	0	1	26
Total	3	1615	10	844	2	18	15	2477

[a] Excludes outbreaks due to chemical contamination.

[b] AGI = Acute gastrointestinal illness of unknown etiology; SRSV = small round structured virus.

Source: Craun 1998.

Tables 5-1 and 5-2 list waterborne disease outbreaks during the 2-year period, 1991–1992. During this period, 14 states and territories reported a total of 41 outbreaks associated with water intended for drinking, including 5 outbreaks attributed to chemical poisoning and 37 outbreaks attributed to infectious agents or unknown causes (Moore et al. 1993, Kramer et al. 1996). Excluding the outbreaks due to chemical contamination, waterborne disease outbreaks in this period caused an estimated 17,386 persons to become ill. Summary statistics for the 1991–1992 period are presented in Tables 5-7 and 5-8. No etiology was established for 25 (68 percent) of the 37 outbreaks attributed to infectious agents or unknown causes, including the largest one reported during this period, in which an estimated 9,847 persons using a filtered surface water supply developed gastroenteritis in a Puerto Rico community. Over three-fourths of the 37 outbreaks were associated with well water sources. Two parasites, *Giardia lamblia* and *Cryptosporidium parvum*, were identified as the etiologic agents for 7 (58 percent) of the 12 outbreaks for which an infectious agent was determined. Five (71 percent) of the outbreaks caused by protozoa were associated with a surface-influenced groundwater source. One outbreak of cryptosporidiosis was associated with filtered and chlorinated surface water.

In six (16 percent) of the 37 outbreaks attributed to infectious agents or unknown causes in 1991–1992, the water source was a lake or river (surface water). All systems associated with these outbreaks had provided chlorination, and four also provided filtration. In the filtered systems, distribution deficiencies were found for one outbreak, no deficiency was identified for one, and the other two were associated with poor filtration of water. During one of these latter outbreaks, the water was also temporarily not chlorinated. One of the unfiltered systems was preparing for an exemption from the filtration required by SWTR, and its raw water quality had been excellent (low turbidity, no coliforms) before the outbreak.

Four of the six surface water systems associated with outbreaks in 1991-1992 were equipped with filtration. In three of these outbreaks, raw water quality had deteriorated because of sewage effluents that were not appropriately diluted as a result of low stream flows during dry weather. During the outbreaks associated with these systems, filtration deficiencies were noted, with elevated turbidity in finished water. For example, although turbidity measurements indicated inefficient operation of the filtration process for the water systems associated with an outbreak in Oregon (May 1992), none of the then-existing EPA water quality regulations were violated by the system during the outbreak period (Moore et al. 1993).

Two outbreaks (6 percent) were associated with contaminated spring water. A giardiásis outbreak occurred when a cross-connection at the water storage tanks allowed contaminated surface water to enter a distribution system. For the other outbreak, which was attributed to *Cryptosporidium*, the presence of algae and diatoms in the drinking water suggested that surface water had entered the spring.

For the 2-year period 1993–1994, 17 states and 1 territory reported a total of 22 outbreaks attributed to infectious agents or unknown causes, as shown in Tables 5-3 and 5-4. Summary statistics for this period are presented in Tables 5-9 and 5-10. Excluding outbreaks attributed to chemical poisoning (e.g., lead, fluoride, copper, and nitrate), waterborne outbreaks in this period caused an estimated 405,273 persons to become ill, including 403,000 from a single outbreak of cryptosporidiosis in Milwaukee, Wisconsin in 1993, the largest outbreak ever documented in the United States, and 2,273 persons from the other outbreaks. No etiologic agent was identified for five (23 percent) of the 22 outbreaks listed. *Giardia* and *Cryptosporidium*

were identified as the principal agents in 10 (59 percent) of the 17 outbreaks for which an etiologic agent other than chemicals was identified. Two outbreaks of cryptosporidiosis occurred in large metropolitan areas (i.e., Milwaukee, WI, and Las Vegas/Clark County, NV), and were significant because they were associated with extended illnesses and deaths of immunocompromised persons in both communities. The waterborne route of transmission of these two outbreaks was not recognized until at least two weeks after their onset (Kramer et al. 1996).

Bacterial agents were also significant in causing waterborne disease in the 1993–1994 period. *Campylobacter jejuni* was implicated in three outbreaks and the following pathogens for one outbreak each: *Shigella sonnei*, *Shigella flexneri*, Non-01 *Vibrio cholerae* (in a U.S. territory; the vehicle was commercially bottled water), and *Salmonella typhimurium* (this outbreak was associated with seven deaths).

Tables 5-9 and 5-10 classify infectious and unknown agent outbreaks occurring from 1993 through 1994 by water system type, etiology, and system deficiency. Eleven (50 percent) of the 22 outbreaks were associated with community systems, 9 (41 percent) with noncommunity systems, and 2 (9 percent) with individual systems. During 1993–1994, outbreaks in noncommunity systems were more likely than those in community systems to be associated with untreated groundwater (5 versus 2 outbreaks). Eight (89 percent) of the 9 outbreaks in the noncommunity systems were associated with well water sources, in comparison with 2 (18 percent) of the 11 community outbreaks in this category.

Of the 22 outbreaks in the period having a known or suspected infectious etiology, 16 (73 percent) occurred in systems using well water or bottled water. For 4 (25 percent) of these systems, all of which used chlorine for disinfection, inadequate or interrupted disinfection was the deficiency identified or suspected (e.g., coliforms, which are chlorine sensitive, were present in tap water). For one of these four systems, deficiencies in filtration also were suspected. No treatment deficiency was identified for the bottled water outbreak in Saipan.

All six outbreaks associated with a lake or reservoir (i.e., surface water sources) were caused by *Cryptosporidium parvum* (3 outbreaks) or *Giardia lamblia* (3 outbreaks). Each of the six systems associated with these six outbreaks provided chlorination, and four also provided filtration. In the filtered systems, deficiencies in the distribution systems were identified for one outbreak, inadequate filtration was identified for one, and no deficiencies were identified for two. In the two unfiltered systems associated with outbreaks of giardiasis, an inadequate contact time with the disinfectant was suspected.

As in previous years, protozoan parasites were the most frequently identified etiologic agents for outbreaks associated with drinking water. An outbreak of cryptosporidiosis in Minnesota also was associated with filtered and chlorinated lake water. *C. parvum* oocysts and *G. lamblia* cysts were detected in lake water but not in finished water.

Two outbreaks of cryptosporidiosis in Washington were associated with well water. The first outbreak, which occurred in Yakima, WA in 1993, was associated with untreated water from a shallow well that was contaminated by surface water. The second outbreak occurred in 1994 near Walla Walla, Washington

(McKinley 1996). For both these outbreaks oocyst structures believed to be *C. parvum* were found in well water samples. In addition, for one of these outbreaks, fecal coliforms were found; for the other, total coliforms were found.

The occurrence of the two outbreaks of giardiasis in New Hampshire (1994) underscores the importance of requiring water systems that use unfiltered surface water to provide an adequate chlorine concentration and contact time (as specified by the SWTR) to inactivate relatively chlorine-resistant organisms such as *Giardia lamblia* (Kramer et al. 1996).

Three of the outbreaks in the 1993-94 period were associated with significant numbers of deaths among sensitive subpopulations in the affected communities. In 1993, distribution system contamination of an unchlorinated municipal water supply in Gideon, Missouri, was determined to be the cause of an outbreak of *S. typhimurium* gastroenteritis that resulted in the deaths of seven nursing home patients (Angulo et al. 1997). The salmonellosis outbreak followed flushing of stagnant water into the system from a water storage tower that was believed to be fecally contaminated by birds. More than 650 persons became ill although a boil water order was issued early in the outbreak (Angulo et al. 1997). During the two years following the 1993 Milwaukee, Wisconsin, outbreak of cryptosporidosis, there were 50 more cryptosporidosis-associated deaths than would have been expected based on prevalence data for the area (Hoxie et al. 1997). Forty-six (85 percent) of these deaths were among persons for whom AIDS was the underlying cause of death (Hoxie et al. 1997). Similarly, in 1994 in Las Vegas/Clark County, Nevada, community exposure to contaminated drinking water from a well-operating, state-of-the-art filtered water supply resulted in a cryptosporidosis outbreak which contributed to the cause of death of at least 20 AIDS patients (Goldstein et al. 1996b).

Craun (1998) reported data on outbreaks associated with water intended for drinking for the years 1995-1996. Information on individual outbreaks (excluding outbreaks caused by chemical contaminants) is presented in Tables 5-5 and 5-6. Table 5-11 presents summary statistics for waterborne outbreaks in 1995-1996. In this two-year period, 8 (53 percent) of the 15 non-chemical outbreaks were attributed to infectious agents, and 7 (47 percent) were attributed to acute gastrointestinal illness (AGI) of unidentified origin. The only reported outbreak of cryptosporidiosis in this period was associated with contamination at the point of use; therefore, this outbreak was not included in the tables (Craun 1998). Two outbreaks of giardiasis were reported in 1995, one associated with high turbidity in a filtered and chlorinated CWS surface water supply in New York; the other associated with consumption of untreated surface water in Arkansas. These two outbreaks accounted for 1459 (59 percent) of the 2477 cases of gastrointestinal illness from non-chemical contaminants in this period.

In 1995-1996, only 3 (20 percent) of the 15 non-chemical outbreaks occurred in CWSs, but accounted for 1615 cases of gastroenteritis (65 percent of the total cases of gastroenteritis reported; see Table 5-11). The number of outbreaks was greater in noncommunity systems (10 outbreaks, equivalent to 66 percent of total outbreaks), but the cases of gastroenteritis reported in noncommunity systems were fewer (844 cases, equivalent to 34 percent of total cases). All 10 outbreaks in noncommunity systems were associated with contaminated groundwater sources (wells or springs); only 1 outbreak in CWSs was associated with a

groundwater supply. Two outbreaks in individual systems (13 percent of total outbreaks) accounted for 18 cases of gastroenteritis (<1 percent of total cases) (Craun 1998).

5.2 OCCURRENCE DATA FOR *CRYPTOSPORIDIUM PARVUM*

5.2.1 Waterborne Outbreaks

In recent years, *Cryptosporidium parvum* has been cited frequently as an etiologic agent of waterborne gastrointestinal illness in the United States (Rose 1997, Craun and Calderon 1996, Kramer et al. 1996, Moore et al. 1993). Cryptosporidiosis associated with these outbreaks accounted for 407,844 reported cases of gastroenteritis (Rose 1997). A single outbreak in Milwaukee, Wisconsin, accounted for 403,000 estimated cases of cryptosporidosis [739 laboratory-confirmed cases (MacKenzie et al. 1994)].

Solo-Gabriele and Neumeister (1996) characterized water supplies associated with U.S. outbreaks of cryptosporidiosis. They determined that almost half of the outbreaks were associated with groundwater (untreated or chlorinated springs and pumped sources), but that the majority of affected individuals were served by filtered surface water supplies (rivers and lakes). Wastewater (sewage) contamination and non-point source runoff from agricultural land were equally important as sources of contamination leading to the outbreaks (Solo-Gabriele and Neumeister 1996).

Raw sewage, surface runoff from livestock grazing areas, septic tank effluent, cattle wastes, treated wastewater, and backflow of contaminated water in the distribution system have been identified as the suspected sources of *Cryptosporidium* contamination of the water supplies in U.S. outbreaks of cryptosporidiosis (Solo-Gabriele and Neumeister 1996). Cattle grazing, feedstock, and, in particular, calves and other young livestock appear to be of greater concern for *Cryptosporidium* than for *Giardia*. Some outbreaks of cryptosporidiosis have been related to upsets (non-routine events, accidents, or failures) in the treatment process of filtered water systems or have occurred on occasions when spikes in turbidity have occurred in those systems. However, little information is available for unfiltered water systems as to whether spikes in raw water turbidity increase the likelihood of having elevated levels of *Cryptosporidium* in the source water. A study is currently underway to collect and analyze the existing *Giardia*, *Cryptosporidium*, and other monitoring data from unfiltered water systems to identify whether there is an increased risk during water quality events (Frey et al. unpublished). Because *Cryptosporidium* cannot easily be controlled with conventional disinfection practices, there is concern about the presence of this organism in the source waters of systems that do not filter (Solo-Gabriele and Neumeister 1996).

5.2.1.1 Outbreaks in Communities Supplied by Surface Water Sources (U.S.)

Documented U.S. outbreaks of cryptosporidosis in populations supplied with drinking water from surface water sources are discussed in this section. Surface water outbreaks in recent years (since 1987) have received considerable study because systems operating in compliance with (at the time) current drinking water

standards for turbidity and other indicators were nevertheless unable to prevent waterborne transmission of the parasite. The outbreaks are discussed in chronological order.

Concentrations of *Cryptosporidium* detected in either source or finished water during U.S. outbreaks are presented in Table 5-12. Although the occurrence of *Cryptosporidium* in United States drinking water supplies has been substantiated by data collected during outbreak investigations, the source and density of oocysts associated with the outbreak have not always been detected or reported. Also, because of limitations and uncertainties of the IFA method used in most studies (see Sect. 3.1), negative results in source or finished water during these outbreaks does not necessarily mean that there were no oocysts in the water at the time of sampling.

Most plants supplying filtered surface water (e.g., Milwaukee, Wisconsin, 1993; Talent, Oregon, 1992; and Carrollton, Georgia, 1987) were experiencing operational deficiencies, high effluent turbidities, or both at the time of the outbreaks. Only one filtered-surface water outbreak, in Clark County, Nevada, 1994, occurred when the filtration system was operating optimally. During groundwater outbreaks involving treated spring or well water, the chlorination systems were apparently operating satisfactorily, with a measurable chlorine residual (Solo-Gabriele and Neumeister 1996).

Bernanillo County, New Mexico – 1986: An epidemiological investigation of gastrointestinal illness associated with untreated surface water established a strong statistical correlation between consumption of the water and 78 confirmed cases of cryptosporidiosis (CDC 1986). Contaminated runoff from livestock grazing areas was identified as a probable source of contamination of the surface water supply in Bernanillo County. Drinking untreated surface water was found to be associated with illness, but recreational exposure (swimming) or attendance at a local day-care center also contributed to the likelihood of being ill, confounding the epidemiological analysis (Rose 1988).

Carrollton, Georgia – 1987: A 4-week outbreak of cryptosporidiosis occurred in Carrollton, Georgia, during January and February 1987, resulting in an estimated 13,000 gastroenteritis cases (Hayes et al. 1989; Rose et al. 1988a,b; Badenoch et al. 1990). *Cryptosporidium* oocysts were the only enteric pathogens identified in the stool samples of 58 of 147 (39 percent) patients with gastroenteritis. The source of the city's drinking water was a river fed by a lake and a number of streams in the vicinity; a sewage discharge existed upstream of the catchment area. Samples of treated water collected in early February contained an average concentration of 0.63 oocysts/L; source water contained 0.08 oocysts/L; and raw sewage from effluent above the catchment area contained 34 oocysts/L. Throughout the period, the plant met EPA water quality standards, treating the water conventionally by coagulation, sedimentation, rapid sand filtration, and disinfection with chlorine. Operational irregularities identified included partial failure of the water treatment system, particularly flocculation of river water abstracted for the municipal supply at a time of high demand; efficiency of filtration was impaired by failure not only of the equipment, but also by procedures used to control water flow through the filters and to monitor turbidity. Other contributing factors included a shutdown of filters when demand was low in December, startup of dirty filters without backwashing, increased precipitation from January 15 to 22, and blockage of a major sewer which led to an overflow of sewage above the water treatment intake. Dye tracer studies showed that the sewage overflow could have reached the water treatment plant via the river

Table 5-12. *Cryptosporidium* and *Giardia* Detection during Outbreaks in Drinking Water Supplies in the U.S.[a]

Year	Location	Population exposed	Number of cases	Source water	Parasite	Oocyst or cyst[b] concentration	Treatment	Suspected cause
1984	Braun Station, TX	5900	2,006[c]	Ground-water	Crypto	None detected	Chlorination	Sewage-contaminated well
1987	Carrollton, GA	32,400	12,960	River	Crypto	Mean = 63 oocysts/100 L Max = 220 oocysts/100 L	Conventional[h]	Treatment deficiencies[i]
1991	Reading, PA		551[d]	Ground-water	Crypto	None reported	Chlorination	Treatment deficiencies[i]
1992	Zephyr Cove, NV		80[b]	Lake	*Giardia*	500 cysts/100 L[b]		Inadequate disinfection[b]
199?	Jackson County, OR	160,000	3,000[d,j]	Spring/ river	Crypto	0.03 oocysts/100 L (confirmed) (range 0.2-0.52 probable)	Chlorination/ package filtration plant	Treatment deficiencies[i]
1993	Milwaukee, WI	1,600,000	403,000[e]	Lake	Crypto	6.7 and 13.2 oocysts/100 L[f] 0.7 and 2.6 oocysts/100 L	Conventional[h]	Treatment deficiencies[i]
1993	Rapid City, SD		7[e]	Well	*Giardia*	32 cysts/100 L	Untreated	Untreated[b]
1993	Idyll Whyle, PA		20[e]	Well	*Giardia*	2 cysts (volume unknown)[f]	Sand filtered, chlorinated[f]	Sewage[k]
1993	Yakima, WA		7[e]	Well	Crypto	Quantity not reported		Untreated
1993	Cook County, MN		27[e]	Lake	Crypto	None detected in filtered water	Filtered, chlorinated	Unknown

Table 5-12 (continued)

Year	Location	Population exposed	Number of cases	Source water	Parasite	Oocyst or cyst[b] concentration	Treatment	Suspected cause
1993	Las Vegas, NV		103[c]	Lake	Crypto	None detected	Conventional	Unknown
1994	Mountain City, TN		304	Spring and reservoir	*Giardia*	58,200 cysts/100 L[b]		Cross connection[h]
1994	Walla Walla, WA		134[e]	Well	Crypto	Quantity not reported		Sewage contamination
1995	Montgomery County, NY	20,700	1,449[g]	Lake	Giardia	None detected	Filtered and chlorinated	Heavy rains, high turbidity

[a] Adapted from Rose et al. 1997a.
[b] From Craun 1996.
[c] D'Antonio et al. 1985.
[d] Moore et al. 1993.
[e] Kramer et al. 1996.
[f] Other references including Rose et al. 1997a and Solo-Gabriele and Neumeister 1996 reported 15,000 cases.
[g] Craun 1998.
[h] Conventional treatment includes coagulation, flocculation, filtration, and disinfection.
[i] Treatment deficiencies include suboptimal performance of one or more steps in conventional treatment.
[j] First set filtered through 0.45 micron filter; second set filtered through 1 micron (nominal) filter. Both sets from ice block with estimated 90% loss of oocysts during freezing (Haas and Rose 1994; cited in Craun 1996).
[k] From John Diehl, personal communication.

in ~6 hours. The source of contamination was never firmly established; it was suspected that shutdown and the restart of the filters without backwashing allowed oocysts to pass into the treated water in sufficiently high numbers to cause the outbreak. By February 11, samples of treated water were negative for oocysts.

Jackson County, Oregon – 1992: A multi-episodic outbreak of cryptosporidiosis was associated with filtered water from two treatment plants (AWWA 1992, Leland et al. 1993). The outbreak was documented mainly by epidemiological evidence (increased numbers of confirmed clinical cases of cryptosporidiosis) during January to June 1992 (most severe May 8 through 31). After the first episode of disease was reported in Medford, Oregon, an analysis of finished water from the community supplier, the Big Butte Springs (discussed further under groundwater sources in Section 5.2.1.2), yielded 1 confirmed and 6 presumptive oocysts in 400 gallons (1,514 L) of water; total coliforms were not detected. A "boil water" notice was issued, and Medford's water system was switched to other sources of treated surface water.

One load of water was trucked in from the Talent, Oregon, water treatment system to customers of the Medford water supply. Talent water (including this truckload) was associated with four subsequent cryptosporidiosis episodes in Jackson County, one of which occurred at a Veterans Administration (VA) domiciliary in White City, Oregon. State health department data on the domiciliary outbreak showed that 203 of 800 (25 percent) patients and 46 of 350 (13 percent) staff persons displayed cryptosporidiosis-like symptoms. A case control study of 26 ill staff members and 32 non-ill controls suggested that the staff who consumed trucked Talent water were 23 times more likely to have developed cryptosporidiosis-like symptoms than the controls. Two other episodes associated with the trucked Talent water were similar to the VA Domiciliary and were consistent with transmission from a single source. A fourth outbreak on May 16, 1992, occurred at a wedding party in Talent attended by 60 persons (AWWA 1992). This outbreak was associated with the Talent water supply, despite the absence of *Cryptosporidium* in punch made from the water (AWWA 1992). There was some epidemiological evidence that immunity may have occurred as a result of endemic exposure to *Cryptosporidium*; none of the 17 Talent residents who attended the wedding developed cryptosporidiosis-like symptoms; however, 13 (32 percent) of 41 non-Talent residents developed such symptoms, and 7 (64 percent) of 11 attendees living outside Jackson County developed symptoms (AWWA 1992).

A water quality investigation revealed that Bear Creek, the water source for one of two rapid-filtration plants serving the Talent community, was significantly affected by agricultural land use and livestock grazing. Bear Creek also receives up to 3 MGD of treated wastewater from the City of Ashland, discharged 5 miles upstream of the Talent intake; the contribution of wastewater to the flow in Bear Creek exceeded 30 percent of flow during the first week of May (Leland et al. 1993). An on-site review of the Talent Bear Creek plant on May 22 revealed poor raw water quality. Turbidity ranged from 1 to 3 NTU in the raw water and was reduced to only 0.4 NTU in the finished water. Filtered water turbidities in the first several months of the year had averaged 1 NTU or greater (Leland et al. 1993). Determination of the oocyst concentration in a filtered water sample taken May 15 was hampered by excessive levels of algae and debris, also indicating poor plant performance (Leland et al. 1993). Based on these data, a boil-water advisory was issued for the City of Talent on May 22. The plant was ordered to begin pre-chlorination, which apparently improved performance so that

the advisory could be lifted May 29. The Big Butte Springs plant also was returned to service. Eight additional analyses did not indicate *Cryptosporidium* oocysts in the spring source (Leland et al. 1993).

A team of experts meeting in August 1992 to analyze the Jackson County outbreak concluded that there was compelling evidence linking the four *Cryptosporidium* outbreaks after May 8 to the Talent water supply (AWWA 1992). No conclusions by the expert team were reached regarding the role, if any, of the Medford water supply in these outbreaks.

Milwaukee, Wisconsin – 1993: This outbreak (March to April 1993) was significant because of the number of cases of cryptosporidiosis (403,000) associated with a filtered water supply. The two water treatment facilities (Howard Avenue and Linwood) serving the population were operating without deficiencies and in compliance with all federal and state regulations at the time of the outbreak (Kaminski 1994; MacKenzie et al. 1994, 1995). Although the city's water treatment method was apparently effective for bacteria and viruses, it was insufficient to inactivate the more resilient *Cryptosporidium* in raw surface water obtained from Lake Michigan (Robertson and Edberg 1997). A dramatic temporary increase in turbidity levels at the Howard Avenue plant was statistically correlated with incidence of gastroenteritis before and during the outbreak (Morris et al. 1996). A boil-water advisory was issued on April 7, and the Howard Avenue plant was closed from April 9 until June 1 (Osewe et al. 1996). In response to the outbreak, the utility also established an internal finished water turbidity standard of 0.1 NTU and more stringent procedures for backwashing filters (Kaminski 1994). Duration and severity of symptoms varied considerably among the affected population. Recurrence of watery diarrhea after apparent recovery was observed among 39 percent of visitors to the area as well as 21 percent of residents with clinical infections (MacKenzie et al. 1995). Over 4,000 hospitalizations (MacKenzie et al. 1994) and over 50 deaths were attributed to the outbreak, representing a 13-fold increase in cryptosporidiosis-associated mortality in the two year period following the outbreak (Hoxie et al 1997).

Haas and Rose (1994) examined oocyst concentrations in ice prepared during the midpoint of the outbreak. Two methods (the ICR method and a membrane filtration method) were used to measure oocysts in the ice and a freeze-thaw loss of up to 90 percent of the oocysts was assumed. Oocysts levels of 0.7 and 2.6 oocysts/100 L (~0.007 and 0.026 oocysts/L) were detected by the ICR method. Membrane filter results were 6.7 and 13.2 oocysts/100 L (~0.067 and 0.132 oocysts/L). Given the freeze-thaw loss, the investigators estimated that the oocyst concentration in finished water during the outbreak ranged from 2.8 to 132 oocysts/100 L (~0.28-13.2 oocysts/L). Haas and Rose also back-calculated dose levels from a dose-response function, and concluded that a drinking water concentration of 0.42-4.5 oocysts/L would have been sufficient to cause the cases of watery diarrhea attributed to the outbreak in March and April of 1993 (Haas and Rose 1994).

Las Vegas/Clark County, Nevada – 1994: The first onset of gastrointestinal illness from this outbreak, reported by Goldstein et al. (1996 a,b), occurred in December 1993; the epidemic extended through June 1994. Although 61 of 78 persons laboratory-confirmed for cryptosporidiosis were human immunodeficiency virus (HIV)-infected adults, the epidemic curves for the onset of illness were similar for immunosuppressed and immunocompetent patients. This outbreak of *Cryptosporidium* contamination in the Las Vegas, Nevada water supply caused widespread illness as well as the deaths of 20 individuals (Goldstein

et al. 1996b). Although Las Vegas obtains 15 percent of its water supply from groundwater, the remainder comes from the Colorado River via Lake Mead to which the outbreak was attributed (Robertson and Edberg 1997).

The Southern Nevada Water System (SNWS) serving the area at the time of the outbreak was a 400 MGD, state-of-the-art facility, drawing water from Lake Mead through an underground tunnel; water was pre-chlorinated, filtered (sand and carbon), and chlorinated again prior to distribution. No deficiencies in treatment or distribution of the water supply, nor any problems with the source, were ever identified (Roefer et al. 1995). Disease cases were evenly distributed among four of five geographical areas served by the plant, supporting the determination of a waterborne outbreak. Because water quality was judged to be good, no boil-water advisory was issued, and persons continued to become infected for 14 weeks after cryptosporidiosis was first suspected (Goldstein et al. 1996 a,b). The outbreak was identified and documented because of the increase in the incidence of cryptosporidiosis in the HIV-infected community and because cryptosporidiosis is a reportable illness in Nevada, so that incidence/prevalence data were readily available to confirm the increase in cases.

In a follow-up evaluation of the outbreak, Roefer et al. (1995) reported that the average turbidity of the raw water from January 1993 to June 1995 (roughly one year before and after the outbreak) was 0.14 NTU, with a high of 0.3 NTU in July 1993, and a low of 0.1 NTU in December 1993 and April 1994. Raw water particle counts in the size range of 2.5 μm to 150 μm averaged 346 particles/mL; the highest raw water count was 910 particles/mL in July 1993 and the lowest was 157 particles/mL in April 1994 (Roefer et al. 1995). Maximum recorded turbidity level for finished water during the outbreak was 0.17 NTU. Although no source of contamination was ever confirmed, presumptive oocysts were identified in lake water and filter backwash samples collected during the follow-up investigation (May 1994 through September 1995). The SNWS voluntarily reduced the effluent water quality particle counts criterion from 20 total particles/mL to 10 total particles/mL (Roefer et al. 1995).

5.2.1.2 Outbreaks in Communities Supplied by Groundwater Probably Under the Direct Influence of Surface Water (U.S., U.K., and Japan)

Outbreaks of cryptosporidosis in drinking water supplies using groundwater as a source indicate that these systems were under the direct influence of surface water (EPA 1994). Because information on such outbreaks is limited, data from other countries [the United Kingdom (U.K.) and Japan] are included in this section. In outbreaks where groundwater is not adequately treated, contamination can occur from sewage overflow and seepage, surface water runoff, streams and rivers, and through limestone and fissured rock (Craun and Calderon 1996). Recent cryptosporidosis outbreaks in groundwater-supplied drinking water in the U.S. and other developed countries include the following:

Braun Station, Texas – 1984: The first documented, but unconfirmed, waterborne outbreak of cryptosporidiosis in the United States occurred in 1984 in Braun Station, a suburb of San Antonio, Texas, which is a community of about 6,000 persons (D'Antonio et al. 1985). The water supply was groundwater from an artesian well, and chlorination was the only method of treatment. Two episodes of gastrointestinal

illness occurred between May and July 1984 as a result of presumed intermittent contamination of the water supply by sewage. Coliform tests of unchlorinated water confirmed that contamination of the water supply occurred between July 2 and 11, 1984 (D'Antonio et al. 1985). During the outbreak investigation, tracer dye introduced into the community's sewage system appeared in the well water. Neither coliform bacteria nor *Cryptosporidium* oocysts were detected in drinking water, but the methods for detection and recovery of oocysts available at the time may have been inadequate. However, clinical evidence (stool samples) collected from infected individuals during the outbreak supported the determination of *Cryptosporidium* as the cause of the outbreak. Although the exact mechanism was not identified, it probably involved a leaking sewage effluent line near the well and/or rapid transmission through channeled limestone to the well from another unidentified source associated with the sewage system (Robertson and Edberg 1997).

A retrospective study of the May 1984 episode revealed acute gastroenteritis (diarrhea and vomiting) in 251 of a total of 346 (72 percent) persons residing in 85 households in the Braun Station area as compared to 3 of a total of 120 (2.5 percent) persons living in 50 households outside Braun Station. This was a significant difference ($p < 0.00001$). Four of six serum specimens collected from ill persons were positive for Norwalk virus antibodies. Serum samples from convalescent persons (number not stated) were negative for virus antibodies.

The July 1984 episode was investigated further to determine the occurrence, causes, and transmission of the disease in the affected population. A total of 117 of 346 (34 percent) immunocompetent persons in the age range 1 to 72 years in 60 Braun Station households had gastroenteritis characterized by diarrhea and abdominal cramps for 1 to 25 days. No correlation was found between infection and geographic (regional) clustering of cases, or between infection and exposure of individuals to farm animals or to swimming in a common community pool. However, among residents taking vacations away from the area between July 1 and 11, 21 percent developed diarrheal illness as compared to 72 percent of those who remained in Braun Station. Forty-seven of 79 residents who became ill had oocysts in their stools; forty of those 47 who were positive for oocysts had diarrhea.

Reading, Pennsylvania – 1991: This outbreak involved a noncommunity well water source associated with an outbreak of cryptosporidosis among persons attending a picnic in August 1991 at Blue Falls Grove in Berks County near Reading, Pennsylvania. Although 551 persons were affected (Moore et al. 1993), others who attended the picnic but did not drink the water did not become ill. The presence of algae, diatoms, and coccidian oocysts in water samples provided evidence that the well was probably under the direct influence of the stream (Moore et al. 1993).

Jackson County, Oregon – 1992: The first of two outbreaks of cryptosporidosis in Jackson County, Oregon, in 1992 (see complete discussion under surface water outbreaks, above) was associated with the Big Butte Springs, a disinfected spring water source that supplied the Medford, Oregon, community of approximately 80,000 persons (Moore et al. 1993). Water samples collected over a 2-week period in February 1992 yielded 1 confirmed and 6 presumptive oocysts in 400 gallons (1,514 L) of water; total coliforms were not detected (Leland et al. 1993). Samples collected during a subsequent 10-week investigation were negative

for *Cryptosporidium*, but were sporadically positive for low levels of algae, total coliforms, and diatoms, suggesting that the spring was under the direct influence of surface water (Moore et al. 1993).

Walla Walla, Washington – 1994: An outbreak of cryptosporidosis affecting residents of a rural community in the Hydro-Nine Irrigation District near Walla Walla, WA was reported in August 1994 (Dworkin et al. 1996, McKinley 1996). At least 15 cases of gastroenteritis were confirmed (fecal specimens positive for *Cryptosporidium*) and at least 71 others were probable (Dworkin et al. 1996, Kramer et al. 1996). The community was supplied by two deep (150 m and 180 m), unchlorinated community wells that tapped the same aquifer. One older well, constructed in 1908, was likely not properly sealed and had a defective casing. This well was contaminated by a cracked irrigation line that carried treated wastewater from the Walla Walla Wastewater Treatment System. The line also carried and potentially leaked pasture runoff that fed back to the irrigation system through several direct connections (McKinley 1996). Another literature source reported that sewage effluent from a weir box only 40 ft from the well was observed leaking into the well and was determined to be the source of the *Cryptosporidium* and other microbes in the well water (Robertson and Edberg 1997). The unchlorinated well water had tested positive for fecal coliforms and *E. coli* and had been disinfected a month before the cryptosporidosis outbreak occurred. Presumptive oocysts were found both in the well water and in the treated wastewater (Dworkin et al. 1996).

Other U.S. Outbreaks: Other small outbreaks associated with GWUDI have been reported. A small outbreak in southeastern Pennsylvania involved an older contaminated private well in a fractured limestone near a contaminated stream and three residential septic systems. A minor outbreak in Oregon involved a spring that apparently became contaminated from adjacent surface water (details unavailable) (Robertson and Edberg 1997).

U.K. Outbreaks in Groundwater

In addition to U.S. outbreaks, several probable GWUDI cryptosporidiosis outbreaks have been reported in the U.K. and one in Japan that provide information on the conditions under which such outbreaks occur. Craun (1996) cited 22 community CWS outbreaks of cryptosporidiosis in the U.K. from 1986-1995, at least 8 of which can be attributed, at least in part, to contamination of groundwater supplies. Other U.K. cryptosporidiosis outbreaks not described here occurred in surface water supplies or private water supplies; small outbreaks have also been attributed to recreational exposure (swimming pools), animal contact during educational farm visits, and one foodborne outbreak (Furtado et al. - submitted).

Isle of Thanet, U.K. – 1991–1992: Craun (1996) reported 160 stool-confirmed cases of cryptosporidiosis from the Isle of Thanet, U.K., in the period December 1990 to January 1991. Five persons were hospitalized. The water source was borehole water supplemented for part of the year by filtered river water for part of the year (29 percent of the flow volume). The borehole treatment is not known. The river treatment plant was used during December 1991, but it is not clear if either river water or borehole water was treated.

Torbay, Devon, U.K. – 1992: Craun (1996) reported a cryptosporidiosis outbreak in Torbay, Devon, England in the period June to November 1992. The number of stool-confirmed cases was 160. The water source was river water supplemented by boreholes in riverside gravel deposits. The river water was filtered, but the borehole water was only chlorinated. One week before the case peak, the turbidity of the borehole source water spiked to 6 NTU. Monthly maximum turbidities of borehole water ranged from 3.6 to 6.2 NTU. The evidence suggests that the oocysts entered either from the river as a result of failure in filtration or through the unfiltered borehole water supply.

Warrington, England, U.K. – 1992–1993: Bridgman et al. (1995) reported an outbreak of cryptosporidiosis in Warrington, England, involving 47 recorded cases of cryptosporidosis between November 1992 and February 1993, most within the first month. The town of Warrington, northwest of London, was supplied by two groundwater sources. Although oocysts were not detected in the water supply, a strong statistical association was observed between drinking unboiled tap water from the wells in the area and the incidence of disease.

Further investigation of the outbreak revealed that a heavy rainfall had occurred at the probable time of waterborne infection. One groundwater source was found to have drained surface water directly from a field containing livestock feces, bypassing natural sandstone filtration. Although the water supply was disinfected following the initial outbreak, a second outbreak of cryptosporidosis occurred. The outbreak ended rapidly when the drinking water supply was replaced with boiled water.

Northumberland Health Authority, U.K. – 1993: Duke et al. (1996) described an outbreak of gastroenteritis in May 1993 in which *Cryptosporidium* and *Campylobacter* were determined to be the etiologic agents. The outbreak occurred in a medieval manor that was connected to several springs via a series of collection chambers and a combination of old and new piping (some cast iron connections dated to the 19th century; others were new in 1986). Water outlets in the residence were protected by filtration (5 μm) and UV lights; however, not all outlet systems were operating optimally at the time of the outbreak, and one outlet was untreated.

The water system was suspected to have been contaminated by infected lambs whose carcasses were found in a collecting chamber of one of the springs. The chamber was previously thought to have been isolated from the system by a plug that had deteriorated over time. During the course of the investigation, high *E. coli* samples in the residential water supply served as the first indicator of fecal contamination. Of 20 fecal samples submitted by the residents for microbiological testing, 5 samples were positive for *Campylobacter* infection, 4 were positive for *Cryptosporidium*, and 2 samples were positive for both pathogens. The overall attack rate for the outbreak was 22 percent of the 200 persons served by the water supply. Thirty percent of the manor's 120 residents were affected.

The lamb carcasses found in the storage chamber had been destroyed before microbiological specimens could be obtained for confirmation of the contamination source. In addition, an inlet to the storage chamber was discovered, and it was also learned that the pasture surrounding the water system had been spread

with slurry five days before the outbreak began and immediately before a period of heavy rainfall. Thus, slurry contamination of the chamber could not be ruled out as the source of contamination (Duke et al. 1996).

Wessex, U.K. – 1993: Morgan et al. (1995) reported a waterborne outbreak of cryptosporidosis from April 1 to May 31, 1993. A total of 64 cases, including 40 primary cases of cryptosporidosis, were reported during the 2-month outbreak. This outbreak, which occurred in the Wessex region of England, had an overall attack rate (estimated ill persons) of less than 1 percent of the population at risk (Craun 1996).

The outbreak was associated with a public water supply drawing from three boreholes. Water abstracted from two of the boreholes had been treated by superchlorination and partial dechlorination; water from a third borehole sited close to a large river was treated by filtration through rapid gravity filters and chlorination. Hydraulic connection of the third borehole with the river was considered unlikely (Morgan et al. 1995).

Three covered reservoirs had been used to store the treated borehole water prior to distribution. Oocysts were detected in a service reservoir that served the town on four occasions (three times in May and once in June 1993) at concentrations that the authors believed too low to be a health hazard (the concentrations of oocysts were not provided). However, the environmental investigation ultimately failed to detect any mechanism for contamination of the water supply. This result suggested the possibility that one or more of the boreholes were contaminated with *Cryptosporidium* (Morgan et al. 1995).

In a survey over a 12-month period in 1993-94, 6 of 605 borehole samples in the U.K. tested positive for *Cryptosporidium* (Quennell and West 1995). The maximum level of oocysts found was 1/L. However, two outbreaks in the U.K. have implicated unfiltered borehole supplies (Furtado et al. – submitted; Morgan et al. 1995).

Cobham, Surrey, U.K.: Lisle and Rose (1995) reported two *Cryptosporidium* outbreaks associated with springs, both in Cobham, Surrey, U.K. One occurred in 1983 and one in 1985. In each case, the source water was filtered and chlorinated.

South and West Devon, U.K. – 1995: The largest waterborne outbreak in the U.K., with 575 confirmed cases, was reported in South and West Devon in the summer of 1995 (Willocks 1997). Another source identified oocysts in the distribution system at 1.1/L in this outbreak (Richardson et al. 1991).

Yorkshire, England, U.K. – 1997: Waterweek (1997), citing an article in Water Bulletin, a U.K. water industry publication, reported that Yorkshire Water issued a boil water order to 4000 persons in a rural community on February 23, 1997. *Cryptosporidium* was detected in finished water. The source for the affected water supply was an aquifer and the treatment method was chlorination. The extent of the illness associated with this occurrence was not reported.

North London, England, U.K. – 1997: Ward (1997) reported that Three Valleys Water (TVW), the supplier of drinking water to 2 million customers in North London, England detected *Cryptosporidium* in a

borehole. Sixteen source, finished water, and distribution system samples were confirmed positive for *Cryptosporidium* (Willocks 1997). The eight TVW wells are located in layers of chalk (soft limestone) that are within 100 to 1,000 m of the Colne River (Waterweek 1997); the wells are naturally recharged and supply 750,000 people. The TVW, North London outbreak (Ward 1997) occurred just after three other outbreaks were investigated in neighboring areas. In each of these outbreaks the only common risk factor was the area of residence. The water supplies to each of these areas were different—some receiving surface water and some receiving borehole supplies.

Willocks (1997) provided additional information on this outbreak of cryptosporidiosis with 345 confirmed cases of cryptosporidiosis in North Thames between February 1 and April 25, 1997. Five cases had dates of onset of diarrhea in the first week of February, nine in the second week, rapidly rising to a peak on March 1, with 24 cases having onset of diarrhea on that day alone. The cases were clustered within four districts. The epidemiological study indicated that of the 345 cases, 244 were primary cases, and 59 were identified as secondary (Willocks 1997).

The population of the four districts received water in varying proportions from Clay Lane treatment works. The Clay Lane treatment works is in Bushey, Hertfordshire, and was originally constructed in 1953. The output from the works averaged 120 million liters per day. The treatment works was served by eight raw water sources sited in the floodplain of the River Colne Valley, penetrating the upper chalk into the middle chalk. Water at Clay Lane was abstracted from wells and boreholes sunk up to 60 m into the underlying chalk. Many of the sources consisted of wells having low level tunnels extending from them. These tunnels (adits) were constructed to intercept areas of locally enhanced groundwater flow, thus increasing the source yields. The Eastbury source consisted of 3 wells and 3 boreholes sunk into the middle chalk, typically 60 m below the ground level. The wells were connected by a series of adits extending to over 1,000 m length at a depth of 50 m. The wells and boreholes were lined to a depth of approximately 20 m. The treatment system consisted of rapid gravity granular activated carbon filtration and disinfection and limited ozonation for pesticide removal (Willocks 1997).

During the outbreak, bottled water was supplied to vulnerable populations such as hospital patients. Boil water orders were rescinded on March 19, 1997 after water samples were negative of oocyst contamination for five consecutive days (Waterweek 1997). Maximum detected levels in raw water were 3 oocysts/15 L and 3 oocysts/10 L in treated water, with a mean oocyst concentration in 16 positive samples of 30 oocysts/100 L. *Cryptosporidium* oocysts were found in one borehole source, the combined raw water works, and the treated water and in the distribution system. TVW investigated the influence of the river on the boreholes. Hydrogeological investigations and study of the catchment area have not revealed how oocysts entered the boreholes (Willocks 1997).

Outbreak in Groundwater in Japan

Ojose, Saitama, Japan – 1996: Hirata and Hashimoto (1997) reported an outbreak of 8,705 cases of cryptosporidiosis associated with a 2-m-deep infiltration gallery in a river bed. Treated wastewaters from two small wastewater treatment plants discharged into the river about 400 m and 1220 m upstream. The

drinking water was treated by coagulation, sedimentation, rapid sand filtration, and chlorination, but no coagulant was added during low turbidity periods. *Cryptosporidium* was found in tap water and other water samples from a receiving tank that was connected with the public water works, and an elevated tank on the roof, a wastewater pits, a soil pit and an artesian spring water tank (Kuroki et al. 1996). This was the first reported outbreak of cryptosporidiosis in Japan distinctly associated with a public water supply.

5.2.2 *Cryptosporidium* Survey and Monitoring Data

Cryptosporidium oocysts have been deleted in wastewater, pristine surface water, surface water receiving agricultural runoff or contaminated by sewage, water for recreational use, and drinking water (Rose 1997, Soave 1995). Rose (1997) summarized historical occurrence data for *Cryptosporidium* in waters in North America by type of water system; these summary data are presented in Table 5-13.

Table 5-13. Detection of *Cryptosporidium* in Surface Water

Water type	Range of percent positives	Range of concentrations (oocysts/L)
Untreated water	67	1–120
Activated sludge effluent	42	0.025–11
Filtered secondarily treated wastewater	42	0.01–0.13
Surface waters	9–100 78.8	0.00–107[a] 5,800[b] 0.02–1.07
Groundwater	5.6	0.004–0.922
Treated drinking water	3.8–40	0.001–0.72
Marine waters influenced by an outfall	62	0.02–0.44

[a] Average of maximum values reported from non-pristine waters.
[b] High levels of oocysts found in waters influenced by agricultural runoff.
References: Chauret et al. 1995, Hansen and Ongerth 1991, Johnson et al. 1995, LeChevallier and Norton 1995, LeChevallier et al. 1991c, Lisle and Rose 1995, Ongerth et al. 1995, Ongerth and Stibbs 1987, Roach et al. 1993, Rose et al. 1996, Rose et al. 1991.
Source: Rose 1997.

Environmental surveys cited here typically involved the collection of a few water samples from a number of sampling locations having different characteristics (e.g., polluted vs. pristine; lakes or reservoirs vs. rivers). Results are presented as they were reported in the original references. The accuracy and precision of the IFA-based analytical methods used for detection of *Cryptosporidium* and *Giardia* in these studies make such data sets unreliable for comparisons and risk assessments; they should be used only to provide a limited view of the potential occurrences and densities of oocysts in source and finished water (Crockett, personal communication, Frey et al. 1997). It also is now recognized that the IFA methods used in these studies can not indicate the absence of oocysts from individual water supplies, or whether the oocysts detected are viable or infective to humans (Frey et al. 1997). Neither does the analytical method define the species of any detected genera of organisms, or the "true" concentration present in water in any sample (Frey et al. 1997).

5.2.2.1 Surface Water Data

Data reported in individual studies on *Cryptosporidium* occurrence in surface water are presented in Table 5-14. *Cryptosporidium* oocysts have been detected raw (source) water and in both filtered and unfiltered drinking water. Ongerth and Stibbs (1987) estimated that the levels of *Cryptosporidium* oocysts reported in river waters ranged from 2 to 112 oocysts/L. Rose et al. (1988a,b) detected *Cryptosporidium* oocysts in 51.4 percent of 111 surface water samples collected in 13 states. However, an earlier study also reported that 91 percent of sewage sampled, 77 percent of the rivers sampled, and 75 percent of lakes sampled contained *Cryptosporidium* oocysts at varying levels (Rose 1988). The oocyst densities in that study ranged from 0.02 to 1.3 oocysts/L (geometric means) in sources ranging from pristine rivers, reservoirs/lakes, to streams/rivers receiving waste water discharges or agriculture pollution (Rose 1988).

Hansen and Ongerth (1991) detected *Cryptosporidium* oocysts in 34 of 35 water samples collected from four locations on two rivers in Washington; concentrations ranged from about 0.2 to 65 oocysts/L overall. Oocyst concentrations were highest in late June when they were influenced by heavy rainfall runoff. They were 10 times higher in samples taken from an uncontrolled watershed than those taken from a controlled public water supply watershed. Similarly, oocyst concentrations were nearly 10 times higher in samples from a downstream area influenced by dairy farming than at upstream locations (Hansen and Ongerth 1991).

Cryptosporidium oocysts have been detected in a number of surveys that included samples of potable water. For example, Rose (1988) reported detecting *Cryptosporidium* in 2 of 4 unfiltered potable water supplies in the western United States. The average concentration of oocysts in the two positive samples averaged 0.006 oocysts/L. Rose et al. (1991) detected 1.7 oocysts/100 L in unfiltered drinking water. LeChevallier et al. (1991c) and LeChevallier and Norton (1995) measured *Cryptosporidium* oocyst and *Giardia* cyst densities in raw water as well as plant effluent samples from selected North American surface water treatment plants during two sampling periods, October 1988 to June 1990, and March 1991 to January 1993. In all, 347 samples from 72 plants (67 locations) were analyzed during the two periods. The ASTM standard method P229 for *Cryptosporidium* was modified slightly to improve oocyst recoveries during this study.

For the 1988 to 1990 sampling period, *Cryptosporidium* oocysts were detected in 87 percent of raw water (intake) samples. Oocyst densities in these samples ranged from 0.07 to 484 oocysts/L (geometric mean = 2.7 oocysts/L). Oocysts were detected in 27 percent of filtered water samples (note: data on *Giardia* occurrence are presented in Section 5.2). Plants where raw water parasite densities were in the higher end of the observed range also had a high probability of detection of cysts and oocysts in treated effluents. For the 1991 to 1993 sampling period, *Cryptosporidium* oocysts were detected in 51.5 percent of raw water (intake) samples; oocyst densities in these samples ranged from 0.065 to 65.1 oocysts/L (geometric mean = 2.4 oocysts/L) (LeChevallier and Norton 1995). LeChevallier and Norton (1995) interpreted their results to be consistent with surveys conducted by Rose (1988a,b).

Table 5-14. Summary of Surface Water Survey and Monitoring Data for *Cryptosporidium* Oocysts

Sample source	Number of samples (n)	Analytical method	Samples positive for *Cryptosporidium* (percent)	Limit of detection[a] (oocysts/L)	Range of oocyst conc. (oocysts/L)	Estimated recovery efficiency[a] (percent)	Mean (oocysts/L)	Estimated viability[a] (percent)	Reference
River	25		100	0.5	2-112	5-20	25.1		Ongerth and Stibbs 1987[b]
River (pristine)	6	WC/IFA	100		2-5800		1920(a)		Madore et al. 1987[b]
Reservoir (polluted)	6		6		0.19-3.0	0.99			Rose 1988
Reservoir (pristine)	6		5		0.01-0.13	0.02			Rose 1988
Surface water	111		51		0.02-1.3				Rose 1988
Impacted river	11		100						Rose et al. 1988a[b]
Lake	20		71		0-22		0.58		Rose et al. 1988b
Stream	19		73.7		0-240		1.09		Rose et al. 1988b[c]
Stream/river	38	WC/IFA	74		<0.001-44		0.66		LeChevallier et al. 1991a
Filtered water	82	WC/IFA	27		0.001-0.48		0.015	8	LeChevallier et al. 1991b[c]
Raw water	85	WC/IFA	87		0.07-484	NA	2.7(g) detectable	32	LeChevallier et al. 1991c
River (pristine)	59	WC/EF	32.2	0.01	NA[b]	29-58 (all samples)	0.29[b]	[b]	Rose et al. 1991a

Table 5-14 (continued)

Sample source	Number of samples (n)	Analytical method	Samples positive for *Cryptosporidium* (percent)	Limit of detection[a] (oocysts/L)	Range of oocyst conc. (oocysts/L)	Estimated recovery efficiency[a] (percent)	Mean (oocysts/L)	Estimated viability[a] (percent)	Reference
River (polluted)	38	WC/EF	73.7	0.01	<0.001-44	29-58 (all samples)	0.66(g)[b]		Rose et al. 1991a
Lake/reservoir (pristine)	34	WC/EF	52.9	0.01	NA	29-58 (all samples)	0.093[b]		Rose et al. 1991a
Lake/reservoir (polluted)	24	WC/EF	58.3	0.01	<0.001-3.8	29-58 (all samples)	1.03[b]		Rose et al. 1991a
River		MF (2 µm)/ IFA		0.04-0.14	0.15-0.45 (pristine) 10-60 (agricultural)	18.6-34.3	0.2 (pristine) 18.3 (agricultural)		Hansen and Ongerth 1991
Protected drinking water supply	6	MF (2 µm)/ IFA	81		0.15-0.42		0.24(g)		Hansen and Ongerth 1991
Pristine river, forestry area	6		100		0.46-6.97		1.62(g)		Hansen and Ongerth 1991
River below rural community in forested area	6		100		0.54-3.6		1.07(g)		Hansen and Ongerth 1991
River below dairy farming agricultural activities	6		100		3.3-63.5		10.72(g)		Hansen and Ongerth 1991
Reservoirs	56		45						Consonery et al. 1992

Table 5-14 (continued)

Sample source	Number of samples (n)	Analytical method	Samples positive for *Cryptosporidium* (percent)	Limit of detection[a] (oocysts/L)	Range of oocyst conc. (oocysts/L)	Estimated recovery efficiency[a] (percent)	Mean (oocysts/L)	Estimated viability[a] (percent)	Reference
Streams	33		48						Consonery et al. 1992
Rivers	37		51						Consonery et al. 1992
Finished water (unfiltered)	6		33		0.001-0.017		0.002		LeChevallier et al. 1992b
Lakes	179	NR	6	NA	0-22.4		0.333 (median)		Archer et al. 1995
Streams	210	NR	6	NA	0-20.0		0.07 (median)		Archer et al. 1995
Finished water	262	WC/IFA	13		0.0029-0.57	8.7-74.7	0.33 (detectable)	35	LeChevallier and Norton 1995
River/lake	262	WC/IFA	52		0.065-65.1		2.4 (detectable)		LeChevallier and Norton 1995
River/lake	147		20		0.3-9.8		2.0		LeChevallier et al. 1995a
River 1	NR		73		0-22.3		1.88(a)		States et al. 1995
River 2	NR		80		0-14.7		1.47(a)		States et al. 1995

Table 5-14 (continued)

Sample source	Number of samples (n)	Analytical method	Samples positive for *Cryptosporidium* (percent)	Limit of detection[a] (oocysts/L)	Range of oocyst conc. (oocysts/L)	Estimated recovery efficiency[a] (percent)	Mean (oocysts/L)	Estimated viability[a] (percent)	Reference
Dairy farm stream	NR		77		0-11.1		1.26(a)		States et al. 1995
Reservoir inlet	60	ICR	5	0.024	0.007-0.024	14	0.012	13	Norton and LeChevallier 1997b
Reservoir outlet	60	ICR	12	0.0.62	0.017-0.31	14	0.081	13	Norton and LeChevallier 1997b
River (polluted)	72	ICR	40		-2.8	16 (range 7-41)	0.24(g)		LeChevallier et al. 1997b
									Stewart et al. 1997a
Source water	NR	ICR	24		0.01-53.9				Swertfeget et al. 1997
First flush (storm event)			35	0.46-417					Stewart et al. 1997a
Filtered (non-storm event)			10	0.03-4.2					Stewart et al. 1997a
Grab (non-storm event)			19	0.03-6.5					Stewart et al. 1997a
Raw water (plant intake)		ICR	63			29	0.58		States et al. 1997a

Table 5-14 (continued)

Sample source	Number of samples (n)	Analytical method	Samples positive for *Cryptosporidium* (percent)	Limit of detection[a] (oocysts/L)	Range of oocyst conc. (oocysts/L)	Estimated recovery efficiency[a] (percent)	Mean (oocysts/L)	Estimated viability[a] (percent)	Reference
Pre-filtration		ICR	29			29	0.12		States et al. 1997a
Finished		ICR	21			29	0.5		States et al. 1997a
Filter backwash		ICR	38				3.3		Stewart et al. 1997a
Reservoirs			37-52	0.43 (max)	0.8-1.4				Okun et al. 1997
Raw water intakes	148		25		0.0004-0.18		0.003		Consonery et al. 1997
Finished water	155		2.5		0.0002-0.008		0.002		Consonery et al. 1997

[a] These data are presented as they were reported in the referenced study.
[b] As cited in Lisle and Rose 1995.
(a) = Arithmetic.
(g) = Geometric.
EF = Epifluorescence microscopy.
ICR = ICR Method.
ICR/WS = Modified ICR well slide.
IFA = Immunofluorescence assay.
MF = Membrane filtration.
NR = Not reported.
WC = Wound cartridge filter.

In general, *Cryptosporidium* oocysts occur in higher concentrations in rivers and streams than in lakes and reservoirs (Rosen et al. 1996; LeChevallier et al. 1991b; Rose et al. 1988a,b). Rosen et al. (1996) compiled and evaluated *Cryptosporidium* and *Giardia* occurrence data provided by 28 U.S. utilities representing 128 unique water sources. The resulting database contained 1,688 observations of *Cryptosporidium* occurrence as well as 1,746 observations of *Giardia* occurrence. The type of source water categories with the highest percent positive results for both protozoa were flowing streams (39.5 percent positive for *Cryptosporidium*; 49.3 percent positive for *Giardia*) and "unknown" sources, while lakes and reservoirs had the lowest percent positive results (24.2 percent positive for *Cryptosporidium*; 19 percent positive for *Giardia*). Oocyst densities are typically but not always higher in raw sewage and treated sewage effluents than in downstream sampling locations. Musial et al. (1987) had estimated that *Cryptosporidium* levels in secondary sewage effluent ranged from 5 to 17 oocysts/L; Madore et al. (1987) found that *Cryptosporidium* levels averaged 5,180 oocysts/L in raw sewage and 1,300 oocysts/L in treated sewage.

LeChevallier and Norton (1995) detected *Cryptosporidium* oocysts in 35 of 262 (13.4 percent) plant effluent samples analyzed between 1991 to 1993. When detected, the oocyst levels averaged 3.3 oocysts/100 L (range = 0.29 to 57 oocysts/100 L). Microscopic evaluation of 77 isolates from finished water samples showed that only 27 (35 percent) contained sporozoites, an indicator of viability. A summary of occurrence data for all samples in filtered effluents for the years 1988 to 1993 showed that 32 (45 percent) of the water treatment plants were consistently negative for *Cryptosporidium*; 24 (34 percent) plants were positive once; and 15 (21 percent) plants were positive for *Cryptosporidium* two or more times between 1988 to 1993. Forty-four (62 percent) of the plants were positive for *Giardia*, *Cryptosporidium*, or both at one time or another (LeChevallier and Norton 1995).

The oocyst recoveries and densities reported by LeChevallier and Norton (1995) for finished water are comparable to results of a broader survey of water supplies(treated and untreated) including pristine (protected watersheds) and polluted (receiving sewage and agriculture discharges) waters (Rose et al. 1991). A total of 188 surface water samples were analyzed from rivers, lakes, or springs in 17 states. While no *Giardia* cysts were found in drinking water samples, *Cryptosporidium* oocysts were detected in 17 percent of 36 samples at levels of 0.5 to 1.7 oocysts/L (Rose et al. 1991). The majority of samples in that survey were obtained from Arizona, California, and Utah (in all 126 samples), and others from eastern states (28 samples), northwestern states (14 samples), southern states (13 samples), mid-western states (6 samples), and 1 sample from Hawaii. Arithmetic average oocyst concentrations ranged from less than 1 to 4,400 oocysts/100 L depending on the type of water analyzed (comparative values for *Giardia* cysts were less than 1 to 140 cysts/100 L). *Cryptosporidium* oocysts were found in 55 percent of the surface water samples at an average concentration of 43 oocysts/100 L (by comparison, *Giardia* cysts were found at 16 percent, 3 cysts/100 L).

Regional surveys of *Cryptosporidium* occurrence have also been conducted. Consonery et al. (1992) reported results of a two-year *Cryptosporidium* monitoring effort in raw and finished water by the Pennsylvania Department of Environmental Resources (PADER). Oocysts were detected in 25 (45 percent) of 56 reservoirs, 19 (51 percent) of 37 rivers, 16 (48 percent) of 33 streams and 2 (33 percent) of 6 springs and wells sampled (see further discussion of groundwater samples in Section 5.2.2.2). Finished water results included detection of oocysts in 2 (14 percent) of clearwell samples and 31 (26 percent) of filter effluent

samples. In all, 47 percent of raw water samples and 25 percent of finished water samples collected between May 1990 and May 1992 were found to be positive for *Cryptosporidium*. Only 21 percent of finished water samples were *Cryptosporidium* positive at plants that received an "acceptable" performance rating, while 32 percent of finished water samples were positive at plants with "unacceptable" performance ratings.

LeChevallier et al. (1995a) analyzed for *Cryptosporidium* and *Giardia* in New Jersey source waters, at 15 sites representing 45 percent of the State water supply. Ten sampling events were conducted over a 1-year period, and 147 samples were analyzed. *Cryptosporidium* oocysts were detected in 20 percent of the samples, with an average concentration of 2.0 oocysts/L (range = 0.3 to 9.8 oocysts/L). The range of detection limits in negative samples (20 to 1536/100 L) was larger than the range of detected values (27 to 976/100 L), indicating the difficulty of reliable detection of protozoa in natural waters.

Archer et al. (1995) reported results for a survey of *Cryptosporidium* and *Giardia* in Wisconsin watersheds. The study followed the major outbreak of cryptosporidiosis in Milwaukee in 1993 (MacKenzie et al. 1994) and was designed to establish the occurrence and distribution of the parasites at intakes for the State's major lake systems used as drinking water sources, and in finished water if the intakes tested positive. Streams in agricultural, urban, and "pristine" watersheds, and wells in the northeast region of the State were also sampled. The authors noted that sampling conditions did not mimic the heavy runoff conditions that occurred in the State just before the Milwaukee outbreak (Archer et al. 1995).

A total of 567 samples were collected by Archer et al. (1995) from November 1993 to May 1995. *Cryptosporidium* oocysts were detected in 29 (5.1 percent) of samples, including the following: 14 (5.9 percent) of 235 stream samples; 10 (5.6 percent) of 179 lake drinking water intake samples; 3 (4.2 percent) of 72 confirmatory finished water samples; 1 of 64 (1.5 percent) wastewater treatment plant effluent samples; and 1 of 17 (5.9 percent) well samples. Results reported for lake drinking water intake samples ranged from 0 to 22.4 oocysts/L *Cryptosporidium* (median = 0.033 oocysts/L) (Archer et al. 1995). Both State studies (New Jersey and Wisconsin) reported source water oocyst densities comparable to ranges reported in various regional studies cited above.

Crabtree et al. (1996) detected *Cryptosporidium* oocysts in public and private cistern systems that are the primary source of drinking water in the U.S. Virgin Islands. The average concentration of oocysts detected in the study was 2.41 oocysts/100 L. *Cryptosporidium* was found in 41 percent of the cisterns in April 1993 and in 59 percent in January 1993.

Swertfeger et al. (1997) have analyzed source and finished water at the Cincinnati, OH treatment facility since 1991. They reported geometric means of 1.85 $\log_{10}$ for presumptively identified oocysts and 2.22 $\log_{10}$ for confirmed identifications when only detects were included in the calculations. When non-detects were included, assuming one oocyst per volume analyzed at the limits of detection, the geometric mean concentrations were 1.0 $\log_{10}$ for presumptives plus non-detects and 0.9 $\log_{10}$ for confirmed plus non-detects. This facility achieved removals of 1.51 $\log_{10}$ for *Giardia* and 1.29 $\log_{10}$ for *Cryptosporidium*.

Norton and LeChevallier (1997b) analyzed the finished water entering and leaving open finished water reservoirs in New Jersey for *Cryptosporidium*, *Giardia*, and other water quality parameters including fecal indicator microorganisms. Open reservoirs are used to store treated drinking water prior to distribution to consumers and these waters may be contaminated with *Cryptosporidium* during storage. Prior to distribution, these waters are redisinfected but not refiltered. Out of 10 samples from each of 6 open reservoirs (i.e., 60 total samples each for *Cryptosporidium* and *Giardia*) operated by 4 water treatment systems, *Cryptosporidium* was found in 3 (5 percent of) inlet samples and 7 (12 percent of) outlet samples. The geometric mean inlet *Cryptosporidium* concentration was 1.2/100 L with a range of 0.7/100 L to 2.4/100 L and the geometric mean outlet concentration was 8.1/100 L with a range of 1.7/100 L to 31/100 L. The authors concluded that the inlet/outlet difference was significant ($p \leq 0.05$) for *Cryptosporidium* even taking into account the variation in the method recovery efficiency (Norton and LeChevallier 1997b).

States et al. (1997) recently reported *Cryptosporidium* densities in river water at two raw water intakes of an urban treatment system serving 500,000 customers. Both river sources flow through agricultural and industrial areas and past sewage treatment plants (States et al. 1997). They detected *Cryptosporidium* oocysts in 63 percent of plant intake samples collected in two years of monthly sampling from each of the two river sources; geometric means of oocyst densities were 31 oocysts/100 L (~0.31 oocysts/L) and 58 oocysts/100 L (~0.58 oocysts/L), respectively. Mean recovery rate of oocysts in all samples was 29 percent (confidence limits 19-39 percent) (States et al. 1997).

The system described by States et al. (1997) employs a chemical treatment center, clarification system (ferric chloride coagulation, flocculation, and settling in primary and secondary basins), a dual-media rapid sand filter, and subsequent disinfection with free chlorine. In comparison, with raw water results, 29 percent of settled water samples (prior to filtration) were positive for oocysts, averaging 12 oocysts/100 L (~0.12 oocysts/L). Oocysts were detected in 21 percent of filtered water samples, averaging 0.5 oocysts/L (~0.005 oocysts/L), effecting an overall 1.49 log removal rate of oocysts during the study (States et al. 1997). The plant typically operated with a filtered water turbidity of 0.1 NTU. The study also monitored oocysts in filter backwash samples from the system; 38 percent of filter backwash samples were positive for oocysts, with a geometric mean of 328 oocysts /100 L (~3.28 oocysts/L) (States et al. 1997).

Stewart et al. (1997a) collected data on *Cryptosporidium* oocyst concentrations in two California watersheds during baseflow and storm events. Five-liter first flush samplers were used to collect samples during storm events and a combination of filtered and unattended and conventional grab samples were used to collect baseflow samples. The highest percentage of positive results were in the first flush samples. The 20 first-flush samples were 35 percent positive for *oocysts*, and oocyst densities in these samples ranged from 46-41,666 oocysts/100 L (~0.41-417 oocysts/L). In comparison, the filtered and grab samples were 10 and 19 percent positive for *Cryptosporidium*, and the observed oocyst densities in the baseflow samples were 3-415 cysts/100 L (~0.03-4.2 oocysts/L) in filtered samples and 3.4-647 oocysts/100 L (~0.03-6.5 oocysts/L) in grab samples (Stewart et al 1997a).

Okun et al. (1997) summarized results of NYDEP monitoring (June 1992-January 1995) of the Catskill, Delaware, and Malcolm Brooks reservoir systems that provide source water to the New York City

water supply, which is unfiltered. The source water, which was sampled prior to chlorination, contained *Cryptosporidium* oocysts in 46, 37, and 52 percent of samples from the Catskill, Delaware, and Malcolm Brooks reservoirs, respectively (Okun et al. 1997). Mean densities of oocysts detected were 1.4, 0.8, and 1.0 oocysts/100 L, densities respectively. Maximum detected oocyst densities in the two-and-one half year monitoring program were 17.3, 15.0, and 43.4 oocysts/100 L for the three reservoirs (Okun et al. 1997). These concentrations in source water were determined to be a threat to the safety of the water supply (Okun et al. 1997).

Consonery et al. (1997) reported protozoa densities in raw and finished water at 284 surface water treatment plants for the period 1985-1996, with an intensive focus on *Cryptosporidium* and *Giardia* since 1994. Of 148 raw water samples analyzed since January 1994, 37 (25 percent) were positive for *Cryptosporidium* at concentrations ranging from 4×10^{-4} to 0.18 oocysts/L. Mean density of oocysts in raw water samples was 0.003 oocysts/L. Of 155 finished water samples, only 4 (2.5 percent) were positive for *Cryptosporidium*. The mean oocyst density in finished water was 0.002 oocysts/L (range 2×10^{-4} to 8×10^{-3} oocysts/L) (Consonery et al. 1997).

5.2.2.2 Data on Groundwater Probably Under the Direct Influence of Surface Water

EPA guidance (EPA 1991) may be used by the primacy States (i.e., those having primary enforcement responsibility for regulation of public water supplies in their states) to determine when groundwater is considered to be GWUDI. Wells, springs, and infiltration galleries all may be evaluated for GWUDI. A microscopic particulate analysis (MPA) which evolved from the analysis of *Giardia* and filtration determinations in groundwater is used to identify GWUDI (EPA 1992.) According to EPA (1991), any detection of a *Giardia* cyst with internal structures in a groundwater sample or any occurrence of a coccidian protozoan or an oocyst-shaped body that has visible internal structures will be assigned a risk score of 20. Wells, springs, and infiltration galleries that receive a score of 20 or greater are at high risk for contamination by *Giardia* and *Cryptosporidium* and should be considered to be GWUDI.

Table 5-15 summarizes available U.S. and U.K. groundwater survey monitoring data for *Cryptosporidium*. Groundwater samples were analyzed by IFA methods or MPA, or both. In addition to the caveats on interpreting IFA method results that were presented in Section 5.2.2, it should be noted that the MPA consensus method is regarded as an evolving method because limited recovery efficiency data are available (EPA 1992). The absence of *Giardia* cysts, coccidia, or other bioindicators indicates a negative sample to the extent of the limit of detection of the analysis performed; it does not ensure that the groundwater source is *Giardia* or pathogen-free (EPA 1992). Conversely, a positive MPA result does not necessarily signify the presence of *Giardia*, *Cryptosporidium*, or other pathogens (EPA 1992).

Table 5-15. Summary of U.S. and U.K. Groundwater Monitoring Data for *Cryptosporidium* Oocysts

Sample source	Number of samples (n)	Samples positive for *Cryptosporidium* (percent)	Limit of detection[a] (oocysts)	Range of positive values (oocysts/L)	Mean (oocysts/L)[b]	Reference
Spring (pristine)	7	28.6		<0.3-13	0.04	LeChevallier et al. 1991a
Groundwater well	17	(1 sample)		1/1175 L		Archer et al. 1995
Vertical wells	149 sites	5[c]	NA	NA		Hancock et al. 1998b
Springs	35 sites	20[c]	NA	NA		Hancock et al. 1998b
Infiltration galleries	4 sites	50[c]	NA	NA		Hancock et al. 1998b
Horizontal wells	11 sites	45[c]	NA	NA		Hancock et al. 1998b
Groundwater sources	199 sites	11	NA	0.002-0.45		Hancock et al. 1998b
Groundwater sources	18	5.61		0.3		Rose et al. 1991
Spring-fed cistern (Potter Co., PA, Nov. 1991)	1	100		Unknown		Conrad 1997; PADEP 1997
Spring (Spring Twp., Center Co., PA, May 1995)	1	100		0.3		Conrad 1997; PADEP 1997
Vertical well Lemont Well #4 (Center Co., PA, Aug. 1992)	6	66.7				Conrad 1997; PADEP 1997
Vertical well (Boggs Twp. Well #1, Center Co., PA, Apr. 1997)	1	100		0.52		Conrad 1997; PADEP 1997
Vertical well (Boggs Twp. Well #1, Center Co., PA, Apr. 1997)	2	50		0.79		Conrad 1997; PADEP 1997
Unconfined chalk borehole, fast recharge, suspected surface water connection (U.K.)	44	0				National *Cryptosporidium* Survey Group 1992
Chalk borehole confined by London Clay (U.K.)	42	0				National *Cryptosporidium* Survey Group 1992

Table 5-15 (continued)

Sample source	Number of samples (n)	Samples positive for *Cryptosporidium* (percent)	Limit of detection[a] (oocysts)	Range of positive values (oocysts/L)	Mean (oocysts/L)[b]	Reference
Partially confined Northern Chalk borehole; very fast recharge, possible contamination of source, sheep graze nearby (U.K.)	34	0				National *Cryptosporidium* Survey Group 1992
Chalk well and adit, urban location with some rural catchment, prone to coliform contamination (U.K.)	46	6.5		0.4-2.6	0.01	National *Cryptosporidium* Survey Group 1992
Chalk borehole, rural catchment with sheep/cattle grazing, prone to coliform contamination (U.K.)	48	4.1		0.07-92.2	0.47	National *Cryptosporidium* Survey Group 1992
Chalk borehole of excellent quality, rural catchment with livestock grazing	44	6.8			0.20	National *Cryptosporidium* Survey Group 1992
GWUDI (U.S. survey, multiple sites)	17	41				Rosen et al. 1996
Infiltration gallery (Huckleberry, PA, June 1991)				Unknown		Fridirici 1997
Vertical well (Douglas Co., OR, Feb. 1996; Feb. 1997)	3	33.3			12	Sebald 1997
Infiltration gallery (Salem, OR, Jan. 1994 through Sept. 1996)	31	35.5		0.3 to 12.7		Salis 1997
Ranney collector (St. Helens, OR, May 1993 through Mar. 1997)	31	3.2	<2.3		4.5	Salis 1997
Well (Marshfield, MA, June 1997)	1	100	0.77		1 (one sample)	Smith 1997
Shallow, vertical well (Braymer Well #4, MO, May 1995)	3	33.3	1.4		0.3	Ledbetter undated

[a] These data are presented as they were reported in the referenced study.
[b] Geometric mean reported unless otherwise indicated.
[c] Number of sites sampled and percent of site positive are reported, rather than the number of samples from each site.
[d] Data compiled for 62 positive samples from all sources in the survey, with outlier data removed from analysis.

Using an IFA method, Rose et al. (1991) found 1 of 18 groundwater sources sampled positive for *Cryptosporidium*. Archer et al. (1995) detected oocysts in 1 of 17 wells tested. LeChevallier et al. (1991) found *Cryptosporidium* oocysts in 2 of 7 spring samples. The range of oocyst concentrations was 0.003 to 0.13 cyst/L with a geometric mean of 0.04 LeChevallier et al. (1991). Rosen et al. (1996) reported 7 of 17 groundwater samples had *Cryptosporidium* oocysts. Consonery et al. (1992) found *Cryptosporidium* oocysts in 2 of 6 groundwater sources (springs or wells) tested in Pennsylvania. Lee (1993) confirmed *Cryptosporidium* occurrence in one of those wells (Lemont #4). This well is 304 ft deep, is drilled into Ordovician medium to thin-bedded limestone and dolomite and is located 95 ft from a stream. Diehl (personal communication) reported *Cryptosporidium* has been found in 7 (of about 400) groundwater sources sampled in Pennsylvania. However, the sources were not identified. In another document, Diehl (1995) identified a well and an infiltration gallery as the sites of two *Cryptosporidium* occurrences. Groundwater monitoring data obtained form various sources show that *Cryptosporidium* has been detected in three vertical wells, one infiltration gallery, one cistern, and one spring in Pennsylvania (Pennsylvania Department of Environmental Protection 1997, Conrad 1997, Fridirici 1997). Known concentrations ranged from 0.3 oocysts per 100 L to 0.79 oocysts per 100 L (see Table 5-15). These data include the Lemont well (Lee 1993) and the infiltration gallery formerly identified by Diehl (1995). The spring also may have been previously identified by Consonery et al. (1992).

Hancock et al. (1998b) reported findings of a survey of groundwater for *Cryptosporidium* at 199 sites using the ICR method. They detected oocysts in 5 percent (7/149) of vertical wells, 20 percent (7/35) of springs, 50 percent (2/24) of infiltration galleries, and 45 percent (5/11) of horizontal wells sampled. Oocyst concentrations ranged from 0.2 to 45 oocysts/100 L in 62 positive samples (outlier data were excluded), with a mean of 5 oocysts/100 L (std = 9), and a median concentration of 2 oocysts/100 L.

In Oregon, *Cryptosporidium* oocysts were detected at three sites:

- In a well drilled into basalt, Sebald (1997) reports that *Cryptosporidium* oocysts were found at 12 oocysts per 100 L. The well is located 80 feet from a river, is 55 feet deep, and is suspected to be linked to the river by a gravel conduit. Microscopic analyses were conducted after close correlations were observed between pH and turbidity values in the river and the well.

- At an infiltration gallery located 15 feet below a river, 11 presumptive *Cryptosporidium* oocyst occurrences have been reported (Salis 1997).

- Salis (1997) also reports the presence of *Cryptosporidium* oocysts (4.5/100 L) in a groundwater sample from a Ranney collector 87 feet deep and 50 feet away from the nearest surface water

Cryptosporidium oocysts also have been detected or indicated in groundwater in Massachusetts, Missouri, and Florida. One presumptive oocyst has been reported in a well in Massachusetts (Smith 1997). In Missouri, one unconfirmed *Cryptosporidium* oocyst (0.3/100 L) was detected in a shallow alluvial well that may have been impacted by flooding (Ledbetter, undated). Harris (personal communication) reported detection of parasitic ova in a well in Florida, indicating the potential for cysts or oocysts to reach that well.

Oocysts have also been detected in groundwater in surveys in the U.K. The National Cryptosporidium Survey Group (1992) detected *Cryptosporidium* in 3 of 6 boreholes tested (8 of 138 samples). Utility companies participating in the U.K. survey had been asked to select a variety of groundwater sources. Another 120 well water samples were negative for *Cryptosporidium*. All three boreholes positive for *Cryptosporidium* were located in chalk aquifers; one was in an urban location, and all were located near rural catchments. The ranges of positive oocyst concentrations in these wells were 0.004 to 0.026, 0.007 to 0.922, and 0.009 to 0.390 oocyst/L, respectively. The arithmetic mean concentration for the boreholes testing positive was 0.23 oocyst/L. Craun (1996) also reported two boreholes and one spring in the U.K. in which *Cryptosporidium* oocysts were detected, but apparently no illnesses or waterborne disease outbreak occurred. One borehole had 0.4 oocyst/100 L; the other borehole had 2 oocysts/100 L. The spring had 286 oocysts/100 L (The National Cryptosporidium Survey Group 1992).

EPA (1997b) reviewed results of MPA determinations in various hydrogeological settings (see Tables 5-16 and 5-17). Protozoa were detected in alluvial, karst, and other groundwater systems greater than 200 ft from surface water sources, and in basalt, alluvial, karst, and fissured bedrock wells at depths exceeding 200 ft (EPA 1997b). Hancock et al. (1998a) performed statistical analyses of another data set [unpublished] to determine if correlations exist between the presence of *Giardia*, *Cryptosporidium*, and other surface water indicators in groundwater. A total of 383 groundwater samples were analyzed for *Giardia*, *Cryptosporidium*, and other microscopic particulates using EPA recommended [MPA and ICR] procedures. The presence of *Giardia* correlated with the presence of *Cryptosporidium* (Hancock et al. 1998a). The presence of both pathogens correlated with the amount [of sample] examined but not with the month of sampling. There was a correlation between source depth and occurrence of *Giardia* but not *Cryptosporidium*.

Hancock et al. (1998a) found no correlation between the distance of the groundwater source from adjacent surface water and detection of either pathogen. However, there was a correlation between designated general risk categories of low, moderate and high and *Cryptosporidium* and *Giardia* occurrence; moreover, the probability of correlation increased with repeat samples. The specific numerical risk values associated with the general categories did not increase the correlation. MPA clearly identified those groundwater sources at low risk for surface influence; however, immunofluorescent assays were necessary to delineate high risk sites (Hancock 1998a).

5.3 OCCURRENCE DATA FOR *GIARDIA*

5.3.1 Waterborne Outbreaks of Giardiasis

Waterborne outbreaks of giardiasis are no longer reported as frequently as they had been (Craun 1996). Reported at a rate of one to four per year in the early 1970's, waterborne giardiasis outbreaks peaked at 20 outbreaks in the two-year period, 1983-1984, but have since declined to seven outbreaks reported during the four-year period from 1991-1994 (Craun 1996). This decline has been attributed to improved water treatment consistent with the 1989 SWTR (Craun 1996). Five outbreaks of giardiasis associated with drinking water in 1993–1994 (Table 5-9), affected an estimated 385 persons and were reported from four states: New

Table 5-16. Protozoa Occurrence Distribution with Well Setback Distance from Nearest Surface Water

Distance from surface water	Samples containing *Giardia* and/or *Cryptosporidium*	Hydrogeologic setting
<100 feet (30 meters)	10 (52.6%)	1 basalt well 4 alluvial wells 1 karst well 1 fissured bedrock well 1 not reported
100 - 200 feet (30 - 61 meters)	6 (31.6%)	3 alluvial wells 2 karst wells 1 not reported
>200 feet (61 meters)	3 (15.8%)	1 alluvial well 1 karst well 1 not reported
Total	19 (100%)	1 basalt well 8 alluvial wells 4 karst well 1 fissured bedrock well 3 not reported

Note: Hydrogeological setting data available for 14 of 17 wells.
Source: EPA 1997b.

Table 5-17. Protozoa Occurrence Distribution with Well Depth

Well depth	Samples containing *Giardia* and/or *Cryptosporidium*	Hydrogeologic setting
≤50 feet (15 meters)	5 (25%)	3 alluvial wells 2 not reported
51 - 100 feet (16 - 30 meters)	9 (45%)	1 basalt well 5 alluvial wells 1 fissured bedrock well 1 not reported
101 - 200 feet (31 - 60 meters)	1 (5%)	1 karst well
>200 feet (61 meters)	5 (25%)	3 karst wells 1 not reported
Total	20 (100%)	1 basalt well 8 alluvial wells 4 karst well 1 fissured bedrock well 4 not reported

Note: Well depth data available for 20 samples with *Giardia* and/or *Cryptosporidium* detections. Hydrogeologic setting available for 14 of 18 wells.
Source: EPA 1997b.

Hampshire (two outbreaks), Pennsylvania (one), South Dakota (one), and Tennessee (one) (Kramer et al. 1996). All giardiasis outbreaks were associated with community water systems; three were associated with surface water supplies and two with well water. Concentrations of cysts detected in water during giardiasis outbreaks in the U.S. are presented in Table 5-12.

5.3.1.1 Surface Water Outbreaks of Giardiasis

From 1971 to 1994, most (78 percent) outbreaks of known infectious etiology in inadequately treated surface water systems were caused by *Giardia* (Craun and Calderon 1997). Coliforms were detected for 11 (92 percent) of the 12 outbreaks of bacterial or unknown etiology but for only 5 (56 percent) of the 9 protozoan outbreaks for which testing was done. For these 9 outbreaks, coliforms were detected for 2 of the 6 systems with chlorinated water but for all 3 with untreated water. For 3 of the 9 outbreaks, water was positive for *Cryptosporidium parvum*; for another 3, it was positive for *Giardia lamblia*. For 1 (14 percent) of the 7 outbreaks with a bacterial etiology, the pathogen *Salmonella* was isolated from the water.

Other surface water outbreaks attributed to *Giardia* (EPA 1994) include Wilkes Barre, Bradford, and Houtzdale, Pennsylvania. Pennsylvania has led the country for a number of years in giardiasis outbreaks, with 7 major giardiasis outbreaks in surface water sources since 1979. This high number of outbreaks is potentially due to a large number of upland reservoirs (Lee 1993). Surface water outbreaks that have occurred since the SWTR became effective in 1990 include the following:

Zephyr Cove, Nevada – March 1992: In an outbreak in a community lake in Nevada, 80 cases of Giardia were reported. The outbreak was attributed to a treatment deficiency (Moore et al. 1993).

Mountain City, Tennessee – March 1994: In Tennessee, a cross-connection between potable and wastewater lines was associated with an outbreak of giardiasis in a correctional facility (Sterling 1995). Potable water was used to cool the seals of a wastewater pump at the facility. A fall in pressure in the potable water system probably caused wastewater to flow back into the potable water line. Subsequently, high concentrations of *Giardia* cysts were detected in tap water (58,200 cysts/100 L [Craun 1996]). Of 424 stool specimens collected from persons in the correctional facility, 110 (26 percent) were positive for *Giardia* and 42 (10 percent) were positive for *Entamoeba histolytica*. A total of 304 cases were reported for this outbreak (Kramer et al. 1996).

New Hampshire – May 1994: There were two outbreaks associated with a community reservoir and a community lake in New Hampshire in May 1994. For the first outbreak in Bartlett, NH, 18 cases were reported, while 36 cases were reported for the second outbreak in New London, NH (Kramer et al. 1996, Craun 1996). These outbreaks were associated with unfiltered, chlorinated surface water (Kramer et al. 1996). For the Bartlett outbreak, water from a reservoir tested positive for *Giardia* (no cyst concentration data available). For the New London outbreak, finished water was positive for total coliforms but not *Giardia*. For both outbreaks, some of the cases were geographically clustered close to the sites where the water was chlorinated (i.e., 28 percent of the former and 50 percent of the latter cases were clustered along proximal

portions of the main lines of the respective distribution systems). This suggested that, for these cases, the chlorine contact time was inadequate (Kramer et al. 1996).

Montgomery County, NY – 1995: A surface water outbreak of giardiasis associated with a CWS in New York resulted in illness in 1449 persons (of a potentially exposed population of 20,700) from December 1995 to February 1996 (Hopkins et al., undated, cited in Craun 1998). This giardiasis outbreak was first attributed to a filtered, chlorinated municipal water system in NY State (Hopkins et al., undated, cited in Craun 1998). Heavy rains were associated with the outbreak (Craun 1998), and although the chlorination system was operating properly, the turbidity readings in the post-filtration water supply exceeded NY State limits before and during the outbreak. *Giardia* cysts were not detected in water samples collected in February 1996 (Hopkins et al., undated, cited in Craun 1998).

5.3.1.2 Giardiasis Outbreaks in Communities Supplied by Groundwater Probably Under the Direct Influence of Surface Water

Outbreaks of giardiasis in drinking water supplies using groundwater indicate that these groundwaters were probably GWUDI. For the period 1965 to 1985, Craun (1990) identified 11 outbreaks associated with a groundwater source. Seven of these were in community systems and 6 of the 11 outbreaks occurred in untreated systems. The remaining systems were unfiltered but were chlorinated. Selected giardiasis outbreaks in groundwater-supplied drinking water are discussed below.

Aspen, Colorado – 1965–1966: The first well-documented waterborne outbreak of giardiasis occurred in Aspen, Colorado in 1965–1966 (Moore et al. 1969). Approximately one-half of the city's water came from a stream and the rest from three wells (Craun 1990). Tracer tests showed that effluent from leaking sewer lines was likely entering the wells. Algae and diatoms were other surface water indicators detected. Approximately 123 cases of suspected giardiasis occurred (Craun 1979).

Park County, Colorado – 1972: This outbreak reported 12 cases of giardiasis associated with ingestion of water from a well in Park County, Colorado. Septic seepage into the well was the likely cause of the outbreak (Craun 1979).

Utah – 1977: In an outbreak at a well located at a Utah campground, 7 cases of giardiasis were reported; untreated well water influenced by surface water was the probable outbreak cause (Craun 1979).

New Hampshire – 1977–1984: Results of a case-control study of 171 giardiasis (Chute 1985, 1987) patients in New Hampshire showed increased risk associated with, among other factors, the use of a shallow well or surface water for individual, household water supply (Craun 1990).

Reno, Nevada – 1982: The Reno outbreak resulted in 342 laboratory confirmed infections. At the time of the outbreak, the city was supplied by both surface water sources (chemically coagulated, settled, and chlorinated, but unfiltered) and groundwater from deep wells. Seventy percent of the water supply was surface water and thirty percent was groundwater (Navin et al 1985). The capture of a beaver infected with *Giardia*

in one of the surface water reservoirs implicated surface water as the source of the outbreak, although other sources and transmission routes were not ruled out (Navin et al. 1985). *Giardia* cyst concentrations in treated water that were equivalent to a density of 3.9 cysts/100 L were reported (Navin et al. 1985).

Colorado – 1986: This outbreak resulting from a treatment deficiency at a well located in a resort area in Colorado, reported 23 cases of giardiasis (CDC 1991).

Danbury, Connecticut – 1987: In this outbreak in Danbury, Connecticut, 120 cases of giardiasis were reported resulting from a distribution system deficiency at a well (Hibler undated, CODHS 1987).

Colorado – 1990: In a community in Colorado served by a spring, 123 cases of giardiasis were reported from aboveground contamination due to land erosion and probably treatment deficiency (Hibler undated, cited in EPA 1994).

California – July 1991: This *Giardia* outbreak occurred in a non-community spring in a recreation area. Distribution system deficiency was attributed as the probable cause leading to 15 reported cases for this outbreak (Moore et al. 1993).

Lemont, Pennsylvania – 1991: Lee (1993) reported 9 confirmed giardiasis cases and several suspected cases associated with two wells in Lemont, Pennsylvania, in a community water system serving 1200 customers. All eight water samples from both wells were positive for *Giardia* cysts. One well is 304 feet deep and 95 feet from the stream; the other well is 323 feet deep and 45 feet from the stream. Both wells are drilled in Ordovician medium to thin-bedded limestones and dolomite that may be solution-enhanced (karst). Green algae, diatoms, and rotifers were other surface indicators found (EPA 1994, Lee 1993).

Pennsylvania – September 1991: This *Giardia* outbreak in Pennsylvania was due to a treatment deficiency in a non-community well located in a park. Thirteen giardiasis cases were reported for this outbreak (Moore et al. 1993).

Idaho – March 1992: Fifteen giardiasis cases were reported from untreated groundwater from a community system well located in a trailer park in Idaho (Moore et al. 1993).

Idyll Whyle, Pennsylvania – 1993: J. Diehl (1997b) reported one confirmed giardiasis case in 1993 associated with dug and drilled wells at a mobile home park in Pennsylvania. The primary drinking water source was a dug well about 12 feet deep that was located adjacent to a stream. The source water was slow sand filtered and chlorinated. Two *Giardia* cysts were found in one of the filter effluent samples. This outbreak of giardiasis in Pennsylvania resulted from sewage contamination. Tests indicated that both *Giardia* and *E. coli* were present in the tap water (J. Diehl 1997a). Another giardiasis groundwater-associated outbreaks was reported by Craun (1996) in McKean, Pennsylvania in 1993.

Rapid City, South Dakota – 1993: The outbreak in South Dakota was attributed to untreated well water contaminated by water from a nearby creek. *Giardia* was found in the well water, and fecal coliforms

were found in both well and tap water. Water quality data (i.e., information as to the presence of coliform bacteria or pathogens) were obtained less than one month after the onset of the outbreak. This outbreak resulted in 7 giardiasis cases with *Giardia* cyst concentrations at 32 cysts/100 L (Craun 1996).

5.3.2 *Giardia* Survey and Monitoring Data

5.3.2.1 Surface Water

Like *Cryptosporidium*, the occurrence of *Giardia* cysts has been documented to be widespread in surface water sources (Table 5-18). As described in Chapter 2 of this report, the distribution of *Giardia* is virtually ubiquitous, because the cysts are excreted by many different types of wild animals, including several with aquatic habitats, such as muskrats and beaver. *Giardia* cysts have been detected in even the most pristine of surface waters (Ongerth 1989).

Rose (1988) detected *Giardia* cysts at a concentration of 0.29 cysts/L in 1 of 6 samples collected at a protected watershed with poor water quality based on standard indicators (turbidity and total coliforms). A second watershed of better water quality based on the indicators contained only 0.006 cysts/L (Rose 1988). *Giardia* cysts were also detected in 12 of 39 samples collected during a biweekly survey of a watershed in the western United States over a 1-year period (Rose et al. 1988 a,b). Sampling was conducted in a lake receiving sewage effluents, and in a river downstream from the lake running through an area where there were a number of cattle pastures. Mean *Giardia* cyst concentrations in the lake were 0.08 cysts/L (range = 0 to 2.22 cysts/L). Means cyst concentrations by season were 0.35, 0.31, 0.007, and 0.001 cysts/L, for the summer, fall, winter, and spring, respectively. *Giardia* cyst concentrations were significantly correlated with *Cryptosporidium* oocyst levels, but there were no significant correlations observed between cyst concentrations and either total or fecal coliforms or turbidity.

Ongerth (1989) documented *Giardia* cyst concentrations in three pristine watersheds in Washington. A membrane filter sampling method was used in the field, with 40 L samples returned to the lab. Cyst recovery efficiencies averaged 21.8 percent ± 6 percent (range = 5 to 44 percent). The cyst concentrations reported were calculated based on the percentage recovery measured in the positive controls, as well as the more routinely applied calculation using the percentage of the microscope slide counted. Ongerth (1989) detected *Giardia* cysts in 94 (43 percent) of 222 samples; cyst concentrations ranged from 0.1 to 5.2 cysts/L. Median cyst concentrations were 0.06, 0.04, and 0.003 cysts/L for the Green, Cedar, and Tolt Rivers, respectively. Cyst concentrations were generally lower in tributaries than in the main stems of the rivers. No seasonal variations could be supported by statistical analyses. Ongerth (1989) concluded that *Giardia* cysts were present continuously at low concentrations in the three rivers.

Rose et al. (1991) reported that *Giardia* cysts were found in 16 percent of the surface water samples at an average concentration of 3 cysts/100 L, while no *Giardia* cysts were detected in the 36 drinking water

Table 5-18. Summary of Surface Water Monitoring Data for *Giardia* Cysts

Sample source	Number of samples (n)	Samples positive for *Giardia* (percent)	Limit of detection[a] (cysts/100 L)	Range of cyst concentration (cysts/100 L)	Estimated recovery rate[a] (percent)	Mean (cysts/100 L)	Estimated viability[a]	Reference
River (downstream from cattle pastures)	39	30.8		0-625		22		Rose et al. 1988
Lake (receiving sewage effluents but upstream of cattle pastures)	39	30.8		0-222		8		Rose et al. 1988
Rivers (pristine: Green, Cedar, Tolt)	222	42.3		10-520	21.8±6	6 (median) 4 (median) 0.3 (median)		Ongerth 1989
Rivers/lakes (polluted and pristine)	38 59 24 34	26 7 33 12		0-625 (poll. river) 0-12 (prist. river) 0-156 (poll. lake) 0-0.5 (prist. lake)		11 (poll. river) 0.35(prist river) 6.5 (poll. lake) 0.5 (prist. lake)		Rose et al. 1991a
Surface water treatment plant source water	85	81.2		4-6600		277	12.8	LeChevallier et al. 1991b
Filtered finished water	83	17.1		0.29-64		4.45	13.3	LeChevallier et al. 1991a
Surface water treatment plant source water	347 (1988-1993)	53.9		2-4380	42.4 (<1 NTU) 50.1 (150 NTU)	200	14.6	LeChevallier and Norton 1995
Source water	262 (1991-1993)	45.0						LeChevallier and Norton 1995

Table 5-18 (continued)

Sample source	Number of samples (n)	Samples positive for *Giardia* (percent)	Limit of detection[a] (cysts/100 L)	Range of cyst concentration (cysts/100 L)	Estimated recovery rate[a] (percent)	Mean (cysts/100 L)	Estimated viability[a]	Reference
Rivers/stream and treated	105							States et al. 1995
Allegheny;		60		12.3-421		42		States et al. 1995
Youghiogheny;		67		43.8-453.7		128.8		States et al. 1995
stream through dairy farm;		69		12.5-29.2		54.9		States et al. 1995
settled water;		13		11.9-70.2		28.9		States et al. 1995
filtered water;		0		0		0		States et al. 1995
filter backwash water		13		14.5-236.8		58.6		States et al. 1995
Lakes/rivers	147	23		40-630		210		Norton et al. 1995
Streams	210	30.9 (47.5 in winter, 25.0-28.8 remainder of the year)		0-2610		38 (median)		Archer et al. 1995
Lakes	179	5.0 (11.3 in spring)		0-125		1.2 (median) (2.6 in spring, median)		Archer et al. 1995
Pristine river	NR	NR		1-5	20	<0.2 (lower reaches)		Ongerth et al. 1995
Recreational use river	NR	NR		1-5	20	0.1 (lower reaches)		Ongerth et al. 1995
Reservoir inlet	60	13	2.4	0.7-2.4	39	1.9	2	Norton and LeChevallier 1997

Table 5-18 (continued)

Sample source	Number of samples (n)	Samples positive for *Giardia* (percent)	Limit of detection[a] (cysts/100 L)	Range of cyst concentration (cysts/100 L)	Estimated recovery rate[a] (percent)	Mean (cysts/100 L)	Estimated viability[a]	Reference
Reservoir outlet	60	15	6.2	1.2-107	39	6.1	2	Norton and LeChevallier 1997
Intakes (river source)		54-63				34-118		States et al. 1997
Pre-filtration		8				29		States et al. 1997
Finished		Not detected						States et al. 1997
Raw water intakes	148	25		0.04-5.7		0.23		Consonery et al. 1997
Finished water	148	Not detected						Consonery et al. 1997
First flush (storm event)	60			25-16,666				Stewart et al. 1997a
Filtered (non-storm)	29			2-119				Stewart et al. 1997a
Grab (non-storm)	19			42-2,428				Stewart et al. 1997a
Reservoirs	29-46			23.4 (max)		0.7-1.3		Okun et al. 1997

[a] These data are presented as reported in the referenced study.

samples. Geometric means ranged from 3 to 4 cysts/100 L in lakes and rivers to 65 cysts/100 L (maximum: 156 cysts/100 L) in polluted lakes. A geometric mean of 0.35 cysts/100 L (maximum: 12 cysts/100 L) were reported for pristine rivers, and for pristine lakes, 0.05 cysts/100 L (maximum: 7/100 L). The geometric mean was 11 cysts/100 L (maximum: 625/100 L) in polluted rivers.

According to Rose (1997), Hibler had previously conducted the most extensive study of waters (more than 4423 samples) for *Giardia* using light microscopy without the aid of antibodies. He found *Giardia* prevalence at 17 to 41 percent in lakes, rivers, and creeks. Only 3 percent of groundwater samples (63 wells) were positive in the Hibler study, and 3.4 percent of the drinking water samples (357 samples postconventional treatment) were positive for *Giardia*. In the Rose et al. (1991) study, no *Giardia* cysts were detected in groundwaters or drinking waters but fewer samples were examined.

LeChevallier et al. (1991b) detected *Giardia* cysts in 69 (81.2 percent) of 85 raw water samples, with a geometric mean of 2.77 cysts/L (range = 0.04 to 66 cysts/L). The samples were taken at well-operated surface water treatment plants in 14 states and 1 Canadian province. LeChevallier et al. (1991c) reported detection of cysts in 17 percent of 83 finished drinking water samples. In a follow-up investigation of these water systems (1991 to 1993), *Giardia* cysts were detected in 118 (45.0 percent) of 262 raw water samples, with a geometric mean of 2.0 cysts/L (range = 0.02 to 43.8 cysts/L)(LeChevallier and Norton 1995).

States et al. (1995) conducted sampling in the vicinity of Pittsburgh, Pennsylvania, in the Allegheny and Youghiogheny Rivers, and the stream passing through a dairy farm. The occurrence, arithmetic mean, and geometric mean of detected *Giardia* cysts in the Allegheny River were 60 percent, 47.8, and 42.0 cysts/100 L, respectively; in the Youghiogheny River they were 67 percent, 128.7, and 128.8 cysts/100 L, respectively; and in the dairy farm stream they were 69 percent, 76.9, and 73.9 cysts/100 L.

Norton et al. (1995) analyzed for *Giardia* in New Jersey source waters, at 15 sites representing 45 percent of the State water supply. Ten sampling events were conducted over a 1-year period, and 147 samples were analyzed. *Giardia* cysts were detected in 23 percent of the samples, with an average concentration of 2.1 cysts/L (range = 0.4 to 6.3 cysts/L).

Ongerth et al. (1995) evaluated the effect of heavy human recreational use on the occurrence of *Giardia* cysts in watersheds in the Olympic Mountains of Washington State. The number of *Giardia* cysts found in water samples ranged from 0.2 cysts/100 L to 3 cysts /100 L and were highest in areas if high human recreational activity (Ongerth et al. 1995).

Archer et al. (1995) reported results for a survey of *Giardia* in Wisconsin watersheds. Sample sites included major lake systems used as drinking water sources; streams in agricultural, urban, and "pristine" watersheds; and wells in the northeast region of the State. A total of 567 samples were collected by Archer et al. (1995) from November 1993 to May 1995. *Giardia* cysts were detected in 65 (30.9 percent) of 210 stream samples, and all 18 stream sites tested positive for *Giardia* cysts at least once during the first year of sampling. Of 179 lake drinking water intake samples, 9 (5.0 percent) were positive for *Giardia*. Results

reported for lake drinking water intake samples ranged from 0 to 125 cysts/100 L (median = 1.2 cysts/100 L). No *Giardia* cysts were found in any of 17 well samples collected from 6 wells in Door County.

Giardia cysts also were detected at levels of concern in drinking water cisterns in the United States Virgin Islands (Crabtree et al. 1996). Over a 1-year sampling period, a total of 45 samples were analyzed for both *Giardia* cysts and *Cryptosporidium* oocysts. The reported average concentration of *Giardia* was 1.09 cysts/100 L with a range from less than 1 to 3.79 cysts/100 L. Of the samples analyzed, 26 percent were positive for cysts. The range of positive analyses for *Giardia* was from 18 percent of the cisterns in January 1993 to 54 percent in July 1992. Because the cisterns are open to the air to collect rainwater, Crabtree et al. (1996) speculated that droppings from birds, rodents, and frogs may have contaminated the cisterns.

Recently, Norton and LeChevallier (1997b) examined the concentrations of cysts at the intake and outlet of 6 open finished water reservoirs in New Jersey operated by 4 water treatment system. They detected *Giardia* cysts in 8/60 (13 percent) of inlet samples and 9/60 (15 percent) of outlet samples over a 1-year period. Open reservoirs are used to store treated drinking water prior to distribution to consumers and these waters may be contaminated with *Giardia* during storage. Prior to distribution, these waters are redisinfected but not refiltered. The geometric mean inlet *Giardia* concentrations was 1.9/100 L with a range of 0.7/100 L to 2.4/100 L and the average outlet concentrations was 6.1/100 L with a range of 1.2/100 L to 24/100 L. The authors concluded that the inlet/outlet difference was not significant ($p \leq 0.05$) for *Giardia* (Norton and LeChevallier 1997b).

States et al. (1997) evaluated *Giardia* densities in river water at the raw water intakes of a major urban treatment system in Pennsylvania, and at intermediate and finished water stages of treatment. Both source rivers feeding this system (the Allegheny and Youghiogheny rivers) flow through agricultural and industrial areas and past sewage treatment plants (States et al. 1995, 1997). In two years of monthly sampling, States et al. (1997) detected *Giardia* in 63 and 54 percent of intake samples from the two river sources; geometric means of cyst densities were 34 cysts/100 L (~0.34 cysts/L) and 118 cysts/100 L (~1.18 cysts/L), respectively. An average 3.26 log removal of *Giardia* cysts was achieved in the system, which includes the following sequential treatment steps: chemical treatment center, clarification system (ferric chloride coagulation, flocculation, and settling in primary and secondary basins), a dual-media rapid sand filter, and disinfection with free chlorine. Only 8 percent of settled water (pre-filtration) samples contained cysts (geometric mean 29 cysts/100 L [~0.29 cysts/L]), and no *Giardia* cysts were detected in filtered samples (States et al. 1997).

Giardia cyst concentrations were measured in two watersheds in relationship to storm events by Stewart et al. (1997a). Percent positive samples and cyst densities were compared in storm-event and baseflow samples. The highest percentage of positive samples were the first flush samples collected during storm events. The first-flush samples were 60 percent positive for *Giardia*. The cyst densities in the first-flush samples ranged from 25 to 16,666 cysts/100 L (~ 0.25-167 cysts/L). In comparison, the filtered and grab samples collected during baseflow conditions were 29 and 19 percent positive for *Giardia*, and the observed cyst densities in the baseflow samples were 2-119 cysts/100 L (~0.02-1.2 cysts/L) and 42-2428 cysts/100 L (~0.42-24.3 cysts/L), respectively (Stewart et al 1997a).

Okun et al. (1997) summarized results of NYDEP monitoring (June 1992-January 1995) of the Catskill, Delaware, and Malcolm Brooks reservoir systems that provide source water to the New York City water supply, which is unfiltered. The source water, which was sampled prior to chlorination, contained *Giardia* cysts in 36, 29, and 46 percent of samples from the Catskill, Delaware, and Malcolm Brooks reservoirs, respectively (Okun et al. 1997). Mean densities of cysts detected were 1.2, 0.7, and 1.3 cysts/100 L, respectively. Maximum detected *Giardia* cyst densities in the two-and-one-half year monitoring program were 9.3, 8.2, and 23.4 cysts/100 L for the three source waters (Okun et al. 1997).

Consonery et al. (1997) reported *Giardia* occurrence levels in raw and finished water in combined findings from the PA Filter Plant Performance Evaluation (FPPE) conducted at 284 surface water treatment plants from 1985-1996, with more intensive analysis of protozoa in the last two years of the study . Since January 1994, 34 (23 percent) of 148 raw water samples analyzed under the program have been positive for *Giardia* cysts, at concentrations ranging from 0.04-5.7 cysts/100 L (~0.004-0.06 cysts/L). Mean density of *Giardia* cysts in positive detect samples of raw water was 0.23 cysts/100 L(~ 0.002 cysts/L) (Consonery et al. 1997). No *Giardia* cysts were detected in finished water during the last two years of the FPPE program (Consonery et al. 1997).

5.3.2.2 Groundwater Occurrence Data for Giardia

Groundwater survey and monitoring data for *Giardia* are presented in Table 5-19. Rosen et al. (1996) reported 3 of 17 samples positive for *Giardia* in sources classified as GWUDI. Hibler (1988) found *Giardia* in 14 percent (5/36) of springs, 5 percent (2/40) of wells, and 31 percent (5/16) of infiltration galleries. As shown in Tables 5-1 through 5.4, 5 of 9 giardiasis outbreaks were associated with groundwater supplies from 1991 through 1994.

Diehl (1997b) reports 11 occurrences of *Giardia* in Pennsylvania. Groundwater monitoring data show that detection sites included two vertical wells, one infiltration gallery, and five springs (Lee 1993, Pennsylvania Department of the Environment 1997, Conrad 1997, Diehl 1997, Fridirici 1997). Concentrations at these sites ranged from 0.3 cysts/100 L to 5.5 cysts/100 L (see Table 5-19). EPA (1994) reports *Giardia* concentrations of 1 cyst/100 gallons from an infiltration gallery, and 3 cysts per 100 gallons in a gravel well that was flooded.

Giardia cysts also were found at three sites in Oregon with *Cryptosporidium* oocyst occurrences previously discussed in Section 5.2.2.2. These three sites were a 55-foot deep basalt well, an infiltration gallery, and a Ranney collector. In the basalt well, *Giardia* cysts were detected at 4 cysts per 100 gallons (Sebald 1997). Two samples collected from the infiltration gallery were found to have presumptive *Giardia* cysts (2/100 L and 22.6/100 L), and the Ranney collector had an unconfirmed occurrence of 9.1 *Giardia* cysts per 100 gallons (Salis 1997).

Hancock et al. (1998b) evaluated the occurrence of *Giardia* in groundwater at 199 sites using the ICR method. *Giardia* cysts were detected in samples from 6 percent (12 of 199) of the sites, including 1 percent (2 of 149) of the vertical wells, 14 percent (5 of 35) of the springs, 25 percent (1 of 4) of the infiltration

Table 5-19. Summary of Groundwater Monitoring Data for *Giardia* Cysts[a]

Sample source	Number of samples (n)	Samples positive for *Giardia* (percent)	Limit of detection[a] (cysts/100 L)	Range of positive values (cysts/100 L)	Mean (cysts/100 L)[b]	Reference
Vertical well	149 sites	1[c]				Hancock et al. 1998b
Spring	35 sites	14[c]				Hancock et al. 1998b
Infiltration gallery	4 sites	25[c]				Hancock et al. 1998b
Horizontal well	11 sites	36[c]				Hancock et al. 1998b
Total groundwater sources	199 sites	6		0.1-120[d]	8	Hancock et al. 1998b
Springs	36	14				Hibler 1988
Wells	40	5				Hibler 1988
Infiltration galleries	16	31				Hibler 1988
Spring (East Haines Twp., Center Co., PA, May 1997)	1	100		0.3		PA DEP 1997; Conrad 1997
Vertical well (Lemont Well #4, Center Co., PA, Feb. 1991 - Aug. 1992)	6	100		1.6-2.9		Lee 1993; PA DEP 1997; Conrad 1997
Vertical well (Lemont Well #5, Center Co., PA, Feb./Mar. 1991)	2	100				Lee 1993; PA DEP 1997; Conrad 1997
Springs (Defiance, PA, Apr. 1994)	1	100		3.7		Fridirici 1997
Infiltration gallery (Huckleberry, PA, June 1991)	1	100		0.3		Fridirici 1997

Table 5-19 (continued)

Sample source	Number of samples (n)	Percent of sites positive for *Giardia* (%)	Detection limit (cysts/100 L)	Range of cyst concentration (cysts/100 L)	Mean (cysts/100 L)	Reference
Springs (Topton Boro, Reading District, PA, Jan. 1993)	1	100		0.3		Fridirici 1997
Spring (Aaronsburg, Spring #2, Center Co., PA, June 1997)	1	100		0.5		Diehl 1997
Spring (Aaronsburg, Spring #4, Center Co., PA, June 1997)	1	100		5.5		Diehl 1997
Vertical well (Douglas Co., OR, Feb 1996, Feb. 1997)	3	33.3		4		Sebald 1997
Infiltration gallery (Salem, OR, Jan. 1994 - Sept. 1996)	31	6.5		0.5-6		Salis 1997
Ranney collector (St. Helens, OR, May 1993 - Mar. 1997)	31	3.2	<2.3	4.5		Salis 1997
Rock well (Byfield, MA, May 1994)	1	100		1		Smith 1997
Caisson well (North Attleboro, MA, Oct. 1992)	1	100		3.1		Smith 1997

[a] These data are presented as they were reported in the referenced study.
[b] Geometric mean reported unless otherwise indicated.
[c] Number of sites sampled and percent of site positive are reported, rather than the number of samples from each site.
[d] Data compiled for 23 positive samples from all sources in the survey, no outlier data in *Giardia* data set.

galleries, and 36 percent (4 of 11) of the horizontal wells. The range of cyst density detected was from <0.1 to 120 cysts/100 L, with a mean density of 8 cysts/100L (std = 25) and a median density value of 2 cysts/100 L (Hancock et al. 1998b). As described in Section 5.2.2, Hancock et al. (1998a) have performed statistical analyses of an unpublished data set (383 groundwater samples), and report a correlation of the detection of *Giardia* in groundwater with the depth of the source sampled. The presence of *Giardia* also correlated with the presence of *Cryptosporidium* in this data set (Hancock et al. 1998a).

In Massachusetts, Smith (1997) reported two *Giardia* occurrences in wells. At one site, 3.1 cysts per 100 gallons were found in a caisson well 24 feet deep and 50 feet away from surface water. At another location, one unconfirmed cyst per 100 L was found in a rock well 90 feet deep and 50 feet away from the nearest surface water body.

5.4 SUMMARY

The occurrence of *Cryptosporidium* and *Giardia* in water intended for drinking has been documented for surface water systems (both raw and finished water) and in groundwater. Monitoring studies have documented increases in oocyst and cyst concentrations in surface water in the first flush after storm events, downstream of dairy farms and sewage treatment effluents, and in pristine reservoirs where finished water is stored prior to distribution . The threat to drinking water from detection or failure to detect oocysts and cysts is difficult to assess because of uncertainties in the monitoring method.

Further evidence of the occurrence of *Cryptosporidium* and *Giardia* in drinking water is found in numerous surveillance reports of waterborne epidemics of cryptosporidiosis and giardiasis and it is possible that endemic disease goes unreported. Infections by these organisms have caused over 400,000 persons in the United States to become reportably ill since 1991. Over 50 immunocompromised individuals have died after contracting cryptosporidiosis during waterborne disease outbreaks since 1991. In 1993-94, two major outbreaks of cryptosporidiosis occurred when the systems were in full compliance with SDWA regulations.

6. OCCURRENCE OF ELEVATED TURBIDITY IN FINISHED WATER

Turbidity has been used as an indicator of drinking water quality and filtration efficiency for many decades. Several reasons exist for controlling turbidity. Suspended particles that cause turbidity can protect pathogens from disinfection by adsorbing and encasing them, thereby reducing or preventing disinfectant contact with the pathogens. In some circumstances, the suspended particles can exert a substantial disinfectant demand, leaving less disinfectant to control pathogens and a greater likelihood of toxic disinfectant byproduct formation. Some particles may have a direct health effect (e.g., pathogens and asbestos fibers). High levels of turbidity can also interfere with analyses for bacteria and protozoa.

The most important reason for monitoring turbidity is to assess the effectiveness of water treatment (see Section 4.4.2). A high turbidity level, whether a short-term spike or one of a more enduring nature, suggests a deficiency in the treatment process that might result in the introduction of pathogens into the distribution system. Very low levels of turbidity, when resulting from a substantial percent removal of turbidity, are also a good indicator of effective protozoan removal during treatment. This section describes the measurement of turbidity and summarizes finished water occurrence data for turbidity, a parameter regulated in the existing SWTR to ensure treatment effectiveness.

6.1 MEASUREMENT OF TURBIDITY

Turbidity is a measure of light scatter resulting from the presence of suspended particles in water. The amount of turbidity depends on the concentration, size distribution, shape, and refractive index of the suspended particles. Turbidity may result from suspended soil and sediment, colloids, or biological materials including pathogens. Methods of measuring turbidity and the use of turbidity measurements to indicate treatment performance are discussed in this section.

6.1.1 Methods

Several nephelometric methods are available to measure turbidity. Turbidimeters commonly consist of a light source to direct light into the sample, a transparent cell to contain the sample to be measured, an optical system to direct scattered light to the detector, a meter to indicate the intensity of scattered light, and light traps to prevent the entrance of stray light into the system. Nephelometers, the most common type of turbidimeters, measure the scattered light at 90° to the incident light. Turbidity is reported in nephelometric turbidity units (NTU). The nephelometer should be able to detect turbidity differences of 0.02 NTU or less in waters with a turbidity of less than 1 NTU (Eaton et al. 1995). A 100-L sample of water with a turbidity of 50 NTU is likely to yield approximately 10 to 30 cc of pelleted particles when the sample is processed through the ICR method.

Benchtop turbidimeters are used to measure turbidity in samples that have been removed from the treatment stream and taken to the turbidimeter; online turbidimeters contain a cell through which the treatment

stream flows. Thus benchtop turbidimeters provide information on discrete samples, whereas online turbidimeters provide more or less continuous data on turbidity.

6.1.2 Use of Turbidity Measurements to Indicate Treatment Performance

Although turbidity may not be a true indicator of pathogen contamination, a very low turbidity level in the treated water is in general a good indicator of effective oocyst and cyst removal (see Section 4.4.2). Establishing that low turbidity in treated water implies effective pathogen removal makes it possible to set treatment performance criteria for turbidity that can protect against pathogens.

6.1.2.1 Correlation of Turbidity Removal with Pathogen Removal

The removal of turbidity during treatment has been shown in many studies to be correlated with removal of pathogens, especially when coagulation has been optimized before filtration. Achieving filtered water turbidities of 0.1 to 0.2 NTU generally corresponds to a turbidity reduction of 1 to 2.5 logs. This level of performance can be accompanied by a reduction of *Cryptosporidium* oocysts by 2.25 to 3 logs (Nieminski and Ongerth 1995) or as high as nearly 6 logs (Patania et al. 1995). Without optimal coagulation, effluent water had a turbidity level below 0.5 NTU, but oocyst removal was at only 1.5 logs (Kelly et al. 1995, Ongerth and Pecoraro 1995), demonstrating the importance of optimal treatment conditions. EPA believes that an optimized filtration system that achieves levels of 0.3 NTU will also achieve a 2-log removal of *Cryptosporidium* oocysts (EPA 1997c).

Samples of source water are generally taken for turbidity measurement at the intake of the treatment facility. Treated water can be sampled at each individual filter, providing a measure of performance of the filter, or at the point where combined filter effluents enter the distribution system, providing a measure of performance of the entire system. Monitoring turbidity at each filter allows a filter whose performance decreases to be identified and taken offline earlier than if combined effluent turbidity is measured. In some cases, effluent turbidity may not present an accurate picture of water treatment; especially when the raw water has low turbidity (e.g., 0.1 to 0.5 NTU) and high alkalinity, chemicals added for coagulation or to condition the water may precipitate in the clear well after filtration, causing elevated turbidity even when sediment and pathogens have been removed from the raw water. EPA has taken the position that in cases where lime softening is practiced and source water turbidities are low, provisions for treatment performance criteria other than direct measurements of turbidity (e.g., acidification of samples to dissolve precipitates) may be appropriate. Microscopic particle analysis (MPA) may be an useful tool to demonstrate adequate treatment performance when influent turbidity is low (Hancock et al. 1996).

6.1.2.2 Current Requirements

The SWTR requires systems using conventional treatment or direct filtration to achieve a turbidity performance criterion of 0.5 NTU for conventional or direct filtration and 1 NTU for slow sand filtration and other filter types. The criterion must be met in 95 percent of the samples taken during each 1-month period,

using a 4-hour sampling interval to sample the combined filter effluent. Systems also may not exceed a maximum total turbidity of 5 NTU.

6.1.2.3 Proposed Requirements

Giardia or *Cryptosporidium* may be present in finished water that meets current SWTR turbidity standards. However, finished water with lower turbidity is more likely to be free of cysts and oocysts. Because *Giardia* cysts and *Cryptosporidium* oocysts are particularly resistant to disinfection, it is extremely important to remove them from drinking water during treatment, necessitating tighter turbidity standards. Therefore, to increase the likelihood that treated water is free of pathogens, the M/DBP Advisory Committee has recommended a turbidity performance criterion of 0.3 NTU in combined filtered water to be met in 95% of samples, with a maximum of 1 NTU, based on samples taken at four-hour intervals.

6.2 OCCURRENCE DATA FOR TURBIDITY

Existing occurrence data for turbidity make it possible to evaluate what fraction of treatment systems are currently likely to be able to meet the proposed criteria. The following sections describe several studies that evaluate turbidity levels, especially the frequency at which treated water that meets current turbidity standards might fail to meet potential new turbidity standards. The majority of samples that met current turbidity standards also meet potential standards of 0.3 NTU in 95 percent of samples, with a maximum of 1 NTU. The M/DBP Advisory Committee has suggested that estimates of the ability of treatment plants to meet a performance standard of 0.3 NTU should be based on the reported ability to achieve a turbidity level of 0.2 NTU to give an adequate safety factor for errors in measurement (EPA 1997c).

6.2.1 State Data

EPA analyzed the annual effluent turbidity data from 86 treatment plants in 11 individual states to assess their current performance. EPA evaluated the number of months in which possible 95th percentile and maximum turbidity levels lower than the current standards were exceeded. The 86 treatment plants analyzed included only those systems serving populations of at least 10,000 people, and only those plants that met the current 95th percentile turbidity standard, 0.5 NTU, and the current maximum turbidity standard, 5 NTU, in all months.

Six states with 47 treatment plants [California (10), Oregon (10), Texas (9), Wisconsin (6), West Virginia (6), and Wyoming (6)] provided multiple values for each day, and five states with 39 plants [Georgia (5), Kansas (9), New Jersey (5), Ohio (12), Rhode Island (6), and Wisconsin (2)] provided daily maximum turbidity measures only. The former data were termed the State 1 data and were considered by EPA to be a more accurate representation of treatment performance because there were more data points. The combined data set, the State 2 data, were considered by EPA to be a good representation of national turbidity occurrence because there was wider geographic representation.

Table 6-1 shows the percentage of plants in the State 1 group in which monthly 95th percentile and maximum values exceeded selected turbidity levels in at least 1, 3, or 6 months out of 12. The table also shows that in 36.2 percent of the plants, the 95th percentile value exceeded 0.2 NTU in at least 1 month of the year, and in 21.3 percent it exceeded 0.3 NTU. Very few plants (less than 2.1 percent) had maximum values exceeding 1 NTU in any month, although over 38 percent exceeded 0.5 NTU in at least 1 month. One plant exceeded 0.5 NTU at least once in at least 6 months of the 12-month period, indicating possible systematic problems with treatment efficacy.

Table 6-1. Number and Percent of Treatment Plants in the State 1 Data Set That Exceeded Turbidity Levels in at Least N Months Out of 12

Turbidity level (NTU)	Statistic	N = 1 Num	N = 1 Pct	N = 3 Num	N = 3 Pct	N = 6 Num	N = 6 Pct
0.1	95th percentile	34	72.3	28	59.6	24	54.1
0.2	95th percentile	17	36.2	9	19.1	2	4.3
0.3	95th percentile	10	21.3	3	6.4	0	0.0
0.4	95th percentile	3	6.4	0	0.0	0	0.0
0.3	Maximum	36	76.6	15	31.9	6	12.8
0.5	Maximum	18	38.3	3	6.4	1	2.1
1.0	Maximum	1	2.1	0	0.0	0	0.0
2.0	Maximum	1	2.1	0	0.0	0	0.0

Table 6-2 shows the percentage of plants in the State 2 group in which monthly 95th percentile and maximum values exceeded selected turbidity levels in at least 1, 3, or 6 months out of 12. The table shows that in 51.2 percent of the plants, the 95th percentile value exceeded 0.2 NTU in at least 1 month of the year, and in 34.9 percent it exceeded 0.3 NTU. Very few plants (less than 7 percent) had maximum values exceeding 1 NTU in any month, although over 40 percent exceeded 0.5 NTU in at least 1 month. One plant exceeded 0.5 NTU at least once in at least 6 months of the 12-month period, indicating possible systematic problems with treatment efficacy.

6.2.2 AWWSC Data

EPA also analyzed annual effluent turbidity data from the American Water Works Service Company (AWWSC). Analyses of the AWWSC data also considered only plants at systems serving populations of at least 10,000 people and that met the current 95th percentile turbidity standard, 0.5 NTU, and the current maximum turbidity standard, 5.0 NTU, in all months. The AWWSC database included annual data for

45 plants in 10 states [California (1), Connecticut (3), Iowa (2), Indiana (6), Maryland (1), Missouri (2), Pennsylvania (24), Tennessee (1), Virginia (2), and West Virginia (3)].

Table 6-2. Number and Percent of Treatment Plants in the State 2 Data Set That Exceeded Turbidity Levels in at Least N Months Out of 12

Turbidity level (NTU)	Statistic	N = 1 Num	N = 1 Pct	N = 3 Num	N = 3 Pct	N = 6 Num	N = 6 Pct
0.1	95th percentile	69	80.2	59	68.6	51	59.3
0.2	95th percentile	44	51.2	29	33.7	15	17.4
0.3	95th percentile	30	34.9	11	12.8	3	3.5
0.4	95th percentile	9	10.5	1	1.2	0	0.0
0.3	Maximum	69	80.2	36	41.9	15	17.4
0.5	Maximum	35	40.7	7	8.1	1	1.2
1.0	Maximum	6	7.0	0	0.0	0	0.0
2.0	Maximum	2	2.3	0	0.0	0	0.0

Table 6-3 shows the percentage of plants in which monthly 95th percentile and maximum values exceeded turbidity standards in at least 1, 3, or 6 months out of 12. The table shows that in 26.7 percent of the AWWSC plants the 95th percentile value exceeded a standard of 0.2 NTU in at least 1 month of the year, and in 13.3 percent it exceeded 0.3 NTU. In only 26.7 percent of the plants the maximum turbidity exceeded 0.5 NTU in any month, with 8.9 percent exceeding 1.0 NTU.

6.2.3 Partnership for Safe Water Data and Recommendations

The Partnership for Safe Water ("Partnership" hereinafter) is a cooperative initiative of organizations representing EPA, American Water Works Association (AWWA), the Association of State Drinking Water Administrators, the Association of Metropolitan Water Agencies, the National Association of Water Companies, and the American Water Works Association Research Foundation (AWWARF). It includes 199 utilities with almost 280 treatment plants that serve approximately 80 million people. Four-hour sampling data were provided for a 12-month period in 1995 and 1996.

Table 6-4 shows the percentage of Partnership plants in which monthly 95th percentile and maximum values exceeded possible turbidity limits in at least 1, 3, or 6 months out of 12. The table shows that in 41.7 percent of the Partnership plants the 95th percentile value exceeded a standard of 0.2 NTU in at least

1 month of the year, and in 19.1 percent it exceeded 0.3 NTU. The maximum turbidity exceeded 0.5 NTU in 27.7 percent of the Partnership plants and 1.0 NTU in 6.8 percent of plants.

Table 6-3. Number and Percent of AWWSC Plants That Exceeded Turbidity Levels in at Least N Months Out of 12

Turbidity level (NTU)	Statistic	N = 1 Num	N = 1 Pct	N = 3 Num	N = 3 Pct	N = 6 Num	N = 6 Pct
0.1	95th Percentile	33	73.3	24	53.3	15	33.3
0.2	95th Percentile	12	26.7	7	15.6	2	4.4
0.3	95th Percentile	6	13.3	1	2.4	0	0.0
0.4	95th Percentile	3	6.7	0	0.0	0	0.0
0.3	Maximum	24	53.3	10	22.2	4	8.9
0.5	Maximum	12	26.7	3	6.7	0	0.0
1.0	Maximum	4	8.9	0	0.0	0	0.0
2.0	Maximum	0	0.0	0	0.0	0	0.0

Table 6-4. Number and Percent of Partnership Plants That Exceeded Possible Turbidity Levels in at Least N Months Out of 12

Turbidity level (NTU)	Statistic	N = 1 Num	N = 1 Pct	N = 3 Num	N = 3 Pct	N = 6 Num	N = 6 Pct
0.1	95th percentile	177	75.3	136	57.9	100	42.6
0.2	95th percentile	98	41.7	51	21.7	27	11.5
0.3	95th percentile	45	19.1	17	7.2	7	3.0
0.4	95th percentile	22	9.4	5	2.1	3	1.3
0.3	Maximum	129	54.9	72	30.6	37	15.7
0.5	Maximum	65	27.7	20	8.5	5	2.1
1.0	Maximum	16	6.8	4	1.7	2	0.9
2.0	Maximum	7	3.0	2	0.9	1	0.4

The Technology Working Group (TWG) of EPA's M/DBP Advisory Committee has pointed out that the turbidity data include only a single year of monitoring and that treatment facilities need to aim at a turbidity

benchmark that is lower than the allowable limit in order to have a treatment safety factor. The TWG recommended that the percentage of treatment facilities meeting a turbidity benchmark of 0.2 NTU should be a reliable estimate of the percentage that could consistently meet a potential standard of 0.3 NTU. The TWG further recommended that the State 2 data could be used to represent facilities serving less than 100,000 consumers, whereas the Partnership data could be used to represent facilities serving more than 500,000 consumers. The State 2 and Partnership data could be averaged to represent facilities serving between 100,000 and 500,000 consumers. Using this classification of the turbidity data, the TWG concluded that, of systems that currently meet a standard of 0.5 NTU in 95 percent of samples, 48.3 percent of systems serving less than 100,000 consumers and 58.3 percent of systems serving more than 500,000 consumers could be expected to consistently comply with a potential standard of 0.3 NTU. The remainder (40 to 50 percent), as well as all of those plants currently failing to meet the performance standard of 0.5 NTU, could be expected to have to make treatment changes in order to comply with a standard of 0.3 NTU.

7. POPULATION PROFILE

The various pathways for exposure to *Cryptosporidium*, *Giardia*, or other microbial enteric pathogens are discussed previously in this report (Chapter 4). These include ingestion of pathogens in drinking water or food; recreational activities leading to ingestion of surface water (e.g., swimming, campers drinking untreated water); direct transmission from animals to humans or from humans to humans. The occurrence of pathogens in drinking water is discussed in Chapter 5. The finished water occurrence of *Cryptosporidium* is not fully characterized, and the ICR data are not yet complete. In addition, the currently available methods cannot detect *Cryptosporidium* reliably at low levels in finished water, and a reliable method to determine pathogen viability has not been developed. Despite the incomplete information, it is confirmed that waterborne outbreaks have occurred in filtered water systems and groundwater systems, and these outbreaks have caused serious illness and deaths of persons in sensitive subpopulations. This chapter describes the populations at potential risk from exposure to waterborne *Cryptosporidium* and *Giardia*.

For detailed discussion on national exposure estimates for *Cryptosporidium*, refer to the "Economic Analysis of M/DBP Advisory Committee Recommendations for the Interim Enhanced Surface Water Treatment Rule," Chapter 6 and Appendix G (October 1997).

7.1 GENERAL POPULATION

Cryptosporidiosis is a common protozoal infection that causes self-limiting diarrhea in immunocompetent humans. For example, in healthy individuals, cryptosporidiosis usually has a duration of less than one month. *Cryptosporidium* has become recognized as a major cause of diarrheal illness both in immunocompetent and immunocompromised hosts throughout the world (Framm and Soave 1997). Giardiasis is often asymptomatic, but infection may also be chronic (Teunis et al. 1997). Infection is the presence of oocyst-positive (DuPont et al. 1995) or cyst-positive stools, with or without symptoms. The most current, although limited, information available concerning giardiasis and cryptosporidiosis in the general population of the United States was reviewed by Craun (1996) and Butler and Mayfield (1996). Additional information is being collected by EPA's Office of Science and Technology.

7.2 SENSITIVE SUBPOPULATIONS

There are a number of sensitive populations that are at greater risk of infection, serious illness (morbidity), or mortality from these pathogens than is the general population. These sensitive populations include children, especially the very young; the elderly; pregnant women; diabetics; the immunocompromised; etc. (Gerba et al. 1996; Fayer and Ungar 1986; EPA 1998a); these subgroups are listed in Table 7-1. This sensitive segment represents almost 20 percent of the population in the United States (Department of Commerce 1991). Because of increasing life spans and increasing numbers of immunocompromised individuals, this number is expected to increase significantly by early in the 21st century. Enteric diseases are most common and devastating among the immunocompromised.

Table 7-1. Total Estimates for Sensitive Population Subgroups

Disease/condition	Year(s) covered	Annual prevalence	Population	Percentage of population
Diabetes	1994	7,765,981	259,633,000[a]	3.0
Osteoporosis	1988-94	3,703,302	251,097,002[b]	1.5
Anemia	1994	4,664,365	259,633,000[a]	1.8
Liver Impairments	1994	594,960	259,633,000[a]	0.2
Cardiovascular Disease	1994	22,279,451	259,633,000[a]	8.6
Hypertension	1994	28,236,261	259,633,000[a]	10.9
Renal Disorders	1994	2,068,952	259,633,000[a]	0.8
Alcoholism (chronic drinking)	1995	5,048,448	Not Available	2.8[c]
Gastric Hyperacidity	1994	6,956,917	259,633,000[a]	2.7
Thyroid Disorders	1994	4,508,780	259,633,000[a]	1.7
Ulcers	1994	4,447,035	259,633,000[a]	1.7
AIDS	1997	64,966	267,645,000[d]	0.02
Cancer Treatment Patients	1992	1,853,795	248,754,000[e]	0.8
Organ Transplant Recipients	1994	17,095	258,932,000[f]	0.01
Immunological Hypersensitivity	1994	40,707,827	259,633,000[a]	15.7
Pregnant Women	1991	6,272,000	129,187,000[g]	4.9
Lactating Women	1990-93	2,247,635	129,813,000[h]	1.7
Occupationally At-Risk	1995	13,891,000	263,034,000[i]	4.4 (biological) 0.8 (chemical)

Source: EPA 1998a.

[a] NCHS 1997a. Note on Rates, Table 1.

[b] NCHS 1997b.

[c] Powell-Griner et al. 1997. Table 4, page 8.

[d] U.S. Bureau of the Census 1996. Table 3, page 9. Middle series resident population projection for 1997.

[e] Collins 1997. Table 45, page 63.

[f] NCHS 1997c. Appendix C, Population Files.

[g] U.S. Bureau of the Census 1996. Table 12, page 14. Female population in the U.S. for the year 1991.

[h] U.S. Bureau of the Census 1996. Table 12, page 14. Annualized female population in the U.S. for the years 1990-1993.

[i] U.S. Bureau of the Census 1996. Table 2, page 8.

Cryptosporidiosis is a serious problem among patients with HIV or AIDS. Patients receiving medications for organ transplantation and cancer patients undergoing chemotherapy are also at significantly greater risk of dying from enteric infections than the general population (Gerba et al. 1996). These data indicate a need for enhanced protection for certain segments of the population who will suffer the most from waterborne pathogens. Because there is currently no consistently proven effective therapy for cryptosporidiosis (Framm and Soave 1997), prevention of infection is critical (Petersen 1992). Giardiasis infection rates do not differ between AIDS patients and the general population (Framm and Soave 1997), but rates of giardiasis in the United States as indicated by hospitalizations were highest among children younger than 5 years of age (Lengerich et al. 1994). Unlike *Cryptosporidium*, there are several effective therapies for treating giardiasis (Rabbani and Islam 1994).

As described in the discussion of the Milwaukee outbreak in Sect. 5.2.1.1, it was reported that more than 50 persons with compromised immune systems were believed to have died prematurely following *Cryptosporidium* infection. In Clark County, Nevada, 61 of the 78 laboratory-confirmed cases of cryptosporidiosis were HIV-infected adults. Of these 61 individuals, 32 died within two months of the outbreak study period, and at least 20 had cryptosporidiosis listed on their death certificate (Craun and Calderon 1996; Goldstein et al. 1996b).

The sensitive status is not limited to individuals with AIDS or patients undergoing chemotherapy. Everyone can be part of a sensitive subpopulation at one time or another in their lives. An individual, throughout his or her lifetime, may pass from sensitive to insensitive population status without any significant warning. In fact, assuming that researchers' claims of acquired immunity are true (see, for example, Frost et al. 1997), the lack of prior exposure or recent exposure to *Cryptosporidium* may actually place an individual in a sensitive subpopulation during traveling (Crockett 1998).

7.2.1 Children

Young children are a vulnerable population subject to infectious diarrhea caused by *Giardia* and *Cryptosporidium* (CDC 1994). Cryptosporidiosis is prevalent worldwide, and prevalence rates are higher in children, especially those younger than age 2, than in adults (Fayer and Ungar 1986). The hospitalization rate for giardiasis in the United States was greatest for children less than 5 years old. Of more than 8,000 hospitalizations for giardiasis in this age group, 36.2 percent occurred among children younger than 1 year old (Lengerich et al. 1994).

These infections often spread from child to child, from children to staff, to family members and other household contacts, and into the community (Pickering et al. 1981; CDC 1994; Tauxe et al. 1986; Weissman et al. 1974). As an indication of the potential size of this sensitive subpopulation, there were more than 21 million children under 6 years of age regularly participating in child care and early education programs in 1994. Of these 21 million, there were approximately 4 million in each of the age groups under 5 (less than 1 year, 1 year, 2 years, 3 years, and 4 years old) and about 1 million in the 5-year-old age group (NCES 1995). In 1997, there were nearly 100,000 licensed child care centers in the United States and its territories, Puerto Rico and Virgin Islands (Children's Foundation 1997).

Child-to-child transmission, particularly among toddlers who are not toilet-trained, may be attributed to their greater degree of personal interaction and to deposition of pathogens on environmental surfaces, which may serve as possible sources for further transmission (Cordell and Addiss 1994). Evidence for secondary transmission of cryptosporidiosis from children to household and other close contacts has been found in many outbreak investigations. Casemore (1990) notes that both sporadic and daycare center cases are often associated with confirmed secondary cases among families. Asymptomatic family contacts of confirmed cases are sometimes found to excrete oocysts in small numbers, thereby providing a hidden reservoir of infection in the community. Stehr-Green et al. (1987) investigated an outbreak of cryptosporidiosis at a daycare center in Florida. Of the 63 family contacts of children who attended the daycare center and were studied, 47 (75 percent) reported a history of diarrhea and 11 (32 percent) of the 34 submitting stool specimens had cryptosporidiosis. Adult household contacts, especially those who changed diapers of infected children, have been shown to be at greater risk of infection than siblings or adults who did not change diapers (Cordell and Addiss 1994; Stehr-Green et al. 1987; Tangermann et al. 1991). Also, the younger children are less likely to have built up immunities or resistance to infection from these pathogens.

Frost et al. (1997) reported on the waterborne cryptosporidiosis outbreak that occurred in Kitchener-Waterloo, Ontario, following that community's conversion from a groundwater source to an oocyst-contaminated surface water supply. During the year following the outbreak, no cryptosporidiosis cases were reported. During the second year, cases occurred primarily in infants and young children. The authors noted that this situation would be expected if contamination of the water confers immunity, resulting in most new cases of illness being limited to young children (or visitors and new residents) (Frost et al. 1997).

Cordell et al. (1997) investigated the impact of the Milwaukee cryptosporidiosis outbreak on child care homes and centers. They noted that the excess risk of director-reported diarrhea in facilities that accepted non-toilet-trained children served as evidence that secondary transmission took place in child care facilities. However, their unpublished data indicate that the presence of asymptomatic *Cryptosporidium* infection did not appear to contribute to an increased risk of diarrhea in infant and toddler rooms (Cordell et al. 1997).

7.2.2 Elderly

There are numerous statements in the literature indicating increased susceptibility of the elderly to illness caused by these enteric pathogens. The American population is aging, and the fastest growing segment will be those over age 85, increasing from 2.3 to 7.3 million from 1980 to 2020. The number of persons over 65 is expected to double from 25 to 50 million (Gerba et al. 1996; Fayer and Ungar 1986).

Infectious diseases are a major concern in the elderly because immune system function declines with age, antibiotic treatment is less effective because of a decrease in physiological function, and malnutrition is more common. Case fatality rates for specific enteric pathogens are 10 to 100 times greater in this group than in the general population (Gerba et al. 1996). As a result, outbreaks of gastroenteritis can be severe in nursing homes because of the high percentage of elderly and the increased potential exposure via person-to-person transmission of that sensitive subpopulation. Of the nearly 1.8 million people who lived in nursing homes in 1990, 1.6 million were 65 or older (U.S. Bureau of the Census 1990). The likelihood of living in a nursing

home increases with age. For example, in 1990 less than 2 percent of those aged 65 to 74 lived in nursing homes compared with about 6 percent of those aged 75 to 84, 19 percent of those aged 85 to 89, 33 percent of those aged 90 to 94, and 47 percent of those aged 95 and older.

More than half of documented deaths from gastroenteritis and hepatitis A illness occur in the elderly in developed countries (Gerba et al. 1996). One factor possibly increasing risk is the level of exposure; Roseberry and Burmaster (1992) reported that daily intake of tapwater increases significantly with age, with persons over 65 ingesting more than twice that of children. However, in epidemiological studies of disease outbreaks, results have been inconsistent. For example, a survey of those affected by the cryptosporidiosis outbreak in Carrollton, Georgia, showed no significant difference in attack rates among age groups (Hayes et al. 1989). Additional data are still needed to confirm the degree of increased susceptibility of the elderly; to understand better the occurrence of the pathogens in drinking water (and food); to determine the effectiveness of current technology for control of pathogens; and to define more accurately to what extent increased susceptibility results from decreasing immune system response, diminished resistance to the physical effects of diarrheal illness and thus reduced recovery rate, or other factors such as increased rate of institutionalization in hospitals or nursing homes with concomitant increased opportunities for exposure.

7.2.3 Immunocompromised Adults

In patients with AIDS and other immunocompromised states, such as those taking certain chemotherapies, *C. parvum* may cause chronic, severe, life-threatening, and often fatal diarrhea. In some cases, protracted and severe diarrhea can cause fluid losses of several liters per day. Symptoms may persist for months, resulting in severe weight loss and mortality rates of 50 percent. Although the prevalence of *C. parvum* in AIDS patients is not certain, chronic cryptosporidiosis was considered an AIDS-defining condition in HIV-infected individuals (Petersen 1992). Recent data suggest that the infection may occur in 15 to 20 percent of patients with AIDS in the United States and in as much as 55 percent of AIDS patients in developing countries (Greenberg et al. 1996; Medical Clinics of North America 1993; Flanigan et al. 1991; Newman et al. 1993). A study of endemic cryptosporidiosis cases in New York City during 1995 showed that 85 percent (397/474) of the reported cases were from HIV positive individuals (Miller et al. 1997). Selik et al. (1997) reported that 95 percent of 420 cryptosporidiosis deaths, 95 percent were HIV infected. These researchers concluded, "HIV infection has so greatly increased the death rate from some opportunistic infections that almost all deaths from them are now due to underlying HIV infection." Several therapeutic drugs have been evaluated in AIDS patients; however, no drug has been consistently effective (Cordell and Addiss 1994). An analysis of potential risk to the immunocompromised subpopulation is being performed by EPA's Office of Science and Technology.

7.3 RECOMMENDATIONS

In their workshop assessing the public health threat associated with waterborne cryptosporidiosis, the Centers for Disease Control and Prevention recommended establishing a task force to provide information to immunocompromised persons that explains how to reduce the risk for cryptosporidiosis, including specific

measures to reduce the risk from waterborne transmission (CDC 1995). These measures may include boiling water, using microstraining personal-use filters, or drinking bottled water.

The Arkansas Department of Health has developed an information packet designed to inform the public, industry professionals, and individuals with weakened immune systems, including those with HIV or AIDS (Barham and Casteel 1995, Arkansas Department of Health 1996). Press releases, brochures, and fact sheets answer common questions about cryptosporidiosis. This type of information is one approach that satisfies the CDC (1995a) Workshop recommendation for information dissemination and education.

A similar, but much more in-depth, response is the AWWARF-sponsored publication, *Cryptosporidium: Answers to Questions Commonly Asked by Drinking Water Professionals*, which addresses issues of public health and awareness, as well as data on *Cryptosporidium* occurrence and public information and management (Frey et al. 1997). The document also includes sources of additional information, such as the Internet and other references and contacts.

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APPENDIX A

PATHOGEN DETECTION METHODS

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PATHOGEN DETECTION METHODS

Analytical methods for *Cryptosporidium* and *Giardia* other than the ICR method are described in the following sections. Methods have been described for the analysis of *Giardia* and *Cryptosporidium* in water and soil. Because most methods apply to both *Giardia* and *Cryptosporidium*, they are described for both pathogens in the following sections. Table A-1 compares a number of the relevant features of the methods. Reviews of analytical methods have appeared in other publications [for example, Erickson (1998), Fricker and Crabb (1998), and Jakubowski et al. (1996)].

METHODS

Membrane Filtration and IFA

Summary. This method (Ongerth and Stibbs 1987, Aldom and Chagla 1995, Nieminski et al. 1995) is a variation of the IFA method in which a large diameter membrane filter rather than a fiber filter is used to concentrate the initial water sample. Cysts and oocysts are removed from the filter and pelleted by centrifugation. They are then resuspended and collected on a membrane filter or in the well of a microscope slide, where they are stained with fluorescent antibody. Stained preparations are examined microscopically for fluorescence, appropriate size and shape, and characteristic morphological structures.

Advantages. As with the ICR and ASTM IFA methods, the limit of detection depends only on the volume examined. That is, the limit of detection is identified as <1 *Giardia* cyst or one *Cryptosporidium* oocyst in the total volume examined, which means that, by increasing the sample volume, the detection limit can be decreased as long as there are not so many interfering particles that observation of oocysts is prevented.

Limitations. All of the limitations of filtration techniques and IFA techniques (i.e., clogging filters by high turbidity samples, interferences from debris or autofluorescing structures, false negatives caused by distortion of diagnostic structures, and expense of equipment) also apply. Also, membrane filters are easily clogged by debris, potentially reducing the limit of detection for slightly turbid waters. Membrane filters in filter holders are cumbersome to transport from the sample site to the laboratory. The membrane slide preparation cannot be cleared which means that visualization of fluorescing objects cannot be performed. This results in inadequate identification procedures. Recovery of oocysts may be little or no better than with cartridge filters; recovery from spiked samples ranged from 5 to 34.3% in two studies (Ongerth and Stibbs 1987, Hansen and Ongerth 1991). Assay is difficult and time-consuming, and it requires trained technicians and expensive microscopes. No formal determination of method detection limit has been done. Not throughly tested with environmental water matrices.

Table A-1. Comparison of Methods and Components for Analysis of *Giardia* and *Cryptosporidium*

Method	Accuracy for environmental samples	Precision for environmental samples	Recovery from environmental media	Identifies species	Measures viability	Measures infectivity	Antibody-based test (potential for cross-reaction and false positives)	Microscope-based identification	Enhancement of microscopic identification (reduced false positives)	Technical skill level
EPA ICR method	Low	Low	Low to fair	No	No	No	Yes	Yes	Yes	High
Membrane filtration and IFA	U	U	LD	No	No	No	Yes	Yes	Yes	High
EPA Method 1622	U	U	Fair to good	No	No	No	Yes	Yes	Yes	High
Zinc sulfate/ Lugol's iodine	Low	Low	Low	No	No	No	No	Yes	No	Low
Immune capture and electrorotation	U	U	Fair	No	LD	LD	Yes	Yes	No	Low
Concentration by calcium carbonate flocculation	LD	LD	Fair to good	NA	NA	NA	No	NA	NA	Low
Immunomagnetic separation	U	U	Fair to good	NA	NA	NA	Yes	NA	NA	Low
Flow cytometry	LD	LD	LD	No	No	No	(1)	Confirmation	(1)	High
Cell culture	U	U	NA	No	Yes	Yes	No	Yes	No	High

Table A-1 (continued)

Method	Accuracy for environmental samples	Precision for environmental samples	Recovery from environmental media	Identifies species	Measures viability	Measures infectivity	Antibody-based test (potential for cross-reaction and false positives)	Microscope-based identification	Enhancement of microscopic identification (reduced false positives)	Technical skill level
Enzyme-linked immunosorbent assay (ELISA)	U	U	NA	No	No	No	Yes	No	No	Low
Polymerase chain reaction (PCR)	U	U	NA	Yes	Potentially	Potentially	No	No	No	Low
Confocal laser scanning microscopy	U	U	NA	No	No	No	No	Yes	Yes	High

U = Unknown.
LD = Limited data.
NA = Not applicable.
(1) = Depends on confirmatory method.

EPA Method 1622

Summary. EPA is validating a method to be used to detect and enumerate *Cryptosporidium* oocysts and *Giardia* cysts in water (EPA 1997d). In this method, a sample of 10 L is filtered with a vortex flow filter, a capsule filter, or a membrane disk filter. Cysts and oocysts are eluted from the filter and are separated from extraneous materials by immunomagnetic separation (IMS). The magnetic beads are removed from the cysts and oocysts, which are then stained with immunofluorescent antibody and a mixture of 4',6-diamidino-2-phenylindole (DAPI). Cysts and oocysts are identified microscopically by fluorescence and are classified on the basis of internal structure and uptake of DAPI.

Advantages. Method 1622 provides positive identification as well as enumeration of *Cryptosporidium* oocysts and will minimize false negatives and confirm the presence of oocysts. The method is described in great detail so that trained analysts should be able to achieve consistent results, and the detection limit depends only on the concentration of interfering particles. Recovery of oocysts by filtration and during centrifugation should be greater than with the ICR method because different conditions are used; acceptance criteria for spiking studies are 15-88 percent for laboratory qualification studies, 14-95 percent for ongoing precision and recovery studies, and 8-127 percent for matrix spike recovery studies (EPA 1997d). Interference from particles should be less than in the ICR method because immunomagnetic separation removes most non-protozoan particles from the sample before microscopic observation. Method 1622 ultimately will be a performance-based method, which means that the methodology that is referenced in the manual can be altered, provided that quality control (QC) tests are conducted and performance specifications are fulfilled.

Limitations. Method 1622 is undergoing validation procedures, so there is not yet a large experience base with the method. As with the ICR method, identification of the immunofluorescent oocysts requires a skilled microscopist and expensive equipment. Each assay requires several hours of sample preparation, and the assay is sufficiently complex that it is unlikely to be performed in the laboratory of a small- or medium-sized water purification plant. Therefore, there are also delays resulting from sample transportation, data review, and reporting. The method also does not distinguish among species of *Cryptosporidium*, nor does it determine viability and infectivity. It is subject to interference from particles in the water, although less than methods that do not include immunomagnetic separation. Because small sample volumes are specified in the protocol, the detection limit for protozoans in clean water should be higher than with the ICR method; method detection limit studies are forthcoming.

Zinc Sulfate/Lugol's Iodine Method

Summary. This method has been used for *Giardia* analysis but appears to be unsuccessful for *Cryptosporidium* oocysts (Standard Methods for the Examination of Water and Wastewater, Volume 18; Eaton et al. 1992). In this method, *Giardia* cysts are concentrated by filtration and flotation on a dense zinc sulfate solution, specific density of 1.2. They are then stained with a non-specific stain (Lugol's iodine) in a zinc sulfate solution. A cover slip is placed at the meniscus of the suspension, and the buoyant material (including cysts) is impinged on the cover slip by centrifugation. Cover slips are then examined microscopically to identify characteristic morphological structures in the stained organisms.

Advantages. Clear visualization of internal features of *Giardia*.

Limitations. Now obsolete, the zinc sulfate method does not appear in the 19th edition of *Standard Methods for the Examination of Water and Wastewater* (Eaton et al. 1995), being replaced by a proposed immunofluorescence method (9711B) that is very similar to the ICR method. Lower *Giardia* recovery than ICR method (LeChevallier et al. 1990). Not used for *Cryptosporidium*. No formal determination of method detection limit has been done.

Immune Capture and Electrorotation

Summary. In this method cysts and oocysts in a concentrated sample are immobilized by antibody-coated magnetic beads and are identified by characteristic rotation in an electric field (Smith et al. 1994, EPA 1995). Samples concentrated by filtration are passed through a stainless steel mesh to which antibody-coated magnetic beads adhere by electromagnetism. Cysts and oocysts are immobilized by binding to the antibody. The magnetic field is then released, allowing the cysts and oocysts to be washed free of the mesh and applied to a microscope slide. A rotating electric field is applied to the specimen under the microscope, and cysts and oocysts are identified by their characteristic rotation rates at various electric field frequencies.

Advantages. The method uses immune capture of cysts and oocysts rather than density gradient centrifugation to isolate them. Therefore, there should be little contamination by debris. The detection technique does not require identification of diagnostic structures by microscopy, but rather depends on observation of rotation in the rotating electric field. Therefore, the method is less difficult and requires less specialized training in microscopy. Because the rate of rotation and the electrical frequency at which rotation occurs depends on surface charge of the cysts and oocysts, it may be possible to distinguish viable from non-viable oocysts and cysts.

Limitations. Limitations of the initial filtration concentration techniques apply. In addition, specialized equipment is required for the generation of the electrical fields. Because the rate of rotation and the electrical frequency at which rotation occurs depends on surface charge of the cysts and oocysts, environmental factors may alter the characteristics of electrorotation. Overall recovery may be similar to that of the ICR method (EPA 1995), but recovery rates have not been systematically determined. Magnification at 400× (as specified by methods) may be insufficient to identify cysts and oocysts. No formal determination of method detection limit has been done. Not throughly tested with environmental water matrices.

Analytical Methods for Soil

Summary. Detection and enumeration of *Giardia* cysts and *Cryptosporidium* oocysts in soil is useful for monitoring protozoan contamination of watersheds and for assessing the potential for contamination of groundwater that may be under the influence of surface run-off. Soil samples placed in suspension in reagent grade water may be analyzed by any of the methods used for analysis of cysts and oocysts concentrated from water. However, these techniques are made difficult by interferences from soil particles that obscure conventionally stained or IFA-stained cysts and oocysts in direct microscopic counts and from non-specific

fluorescence in IFA-aided determinations. *Cryptosporidium* oocysts have been detected and enumerated in soil and sediment samples by a combination of confocal laser scanning and conventional epifluorescence microscopy (Anguish and Ghiorse 1997). Oocysts were stained with fluorescent vital dyes and IFA. They were then examined by conventional and confocal laser-scanning epifluorescence, with the use of a computer to project images simultaneously on two video monitors so the two images could be compared. This method was able to detect *Cryptosporidium* oocysts at a concentration of 520 per gram of soil.

Advantages. Can identify *Giardia* and *Cryptosporidium* in the presence of large quantities of particulate material.

Limitations. Does not identify species nor infectivity. Interferences from soil make analysis difficult. Application of the method requires a large investment in instrumentation and a skilled microscopist. No recovery values are generated.

METHOD COMPONENTS

Calcium Carbonate Flocculation

Summary. Cysts and oocysts can be concentrated from water samples by flocculation in a precipitate formed with calcium chloride, sodium bicarbonate, and sodium hydroxide are added to a sample containing the pathogens (Vesey et al. 1993b; Ho et al. 1995; Shepherd and Wyn-Jones 1995, 1996). When the precipitate is collected and dissolved, cysts and oocysts may be collected and analyzed by a variety of methods.

Advantages. This method appears to allow a higher recovery of cysts and oocysts than filtration methods (Shepherd and Wyn-Jones 1995, 1996) because losses of cysts and oocysts are reduced.

Limitations. Limited by concentration techniques or by volume of sample that can be subjected to flocculation. No formal determination of method detection limit has been done. Not throughly tested with environmental water matrices.

Immunomagnetic Separation (IMS)

Summary. Immunomagnetic separation methods use antibody molecules attached to iron particles or magnetic beads to trap cysts and oocysts in a magnetic field. Cysts and oocysts can then be removed from the magnetic antibody or the magnetic field can be removed, allowing the cysts and oocysts to be collected by centrifugation (Parker and Smith 1994).

Advantages. Removes most of the interfering debris from the suspension of cysts and oocysts, improving conditions for subsequent preparation and analysis. *Cryptosporidium* and *Giardia* IMS kits are commercially available.

Limitations. The method is not sufficiently developed to establish the full range of applicability to low-turbidity and high-turbidity water. No formal determination of method detection limit has been done, but detection limits are being determined in conjunction with Method 1622. Not throughly tested with environmental water matrices.

Flow Cytometry

Summary. Flow cytometry methods allow for both counting and sorting *Giardia* cysts and *Cryptosporidium* oocysts in concentrated samples. Samples may be concentrated by a combination of filtration and centrifugation. The concentrated suspension is reacted with a fluorescent antibody and passed through a flow cytometer which analyzes the size and shape of fluorescent particles. Fluorescent particles meeting the established criteria of size and shape are automatically sorted into a separate container, from which they can be recovered and identified by fluorescence microscopy or PCR (Abbaszadegan et al. 1991; Awad-el-Kariem et al. 1994; Danielson et al. 1995; Hoffmann et al. 1995; Johnson et al. 1995; Vesey et al. 1993a, 1994).

Advantages. Because the sorting function of the flow cytometer separates cysts and oocysts from most of the debris, their identification is easier than with other IFA techniques. The method may be quicker and may have a higher recovery rate than the standard ICR technique (Jakubowski et al. 1996).

Limitations. Clogging of filters by high-turbidity samples and false negatives caused by distortion of diagnostic structures may still occur. The technique requires expensive equipment and highly trained technicians. It may therefore be more suited to a research laboratory than to the analytical laboratory of a treatment facility. No formal determination of method detection limit has been done. Not throughly tested with environmental water matrices.

Cell Culture

Summary. Because *Giardia* and *Cryptosporidium* can infect cells in vitro, cell culture can be used to determine the concentration of infective cysts and oocysts in a concentrated sample. Cultured human cells, such as HCT-8 adenocarcinoma (LeChevallier et al. 1996, Slifko et al. 1997, Upton et al. 1995) or other lines (Upton et al. 1994a,b; Rochelle et al. 1997a) are inoculated in culture flasks or microtiter plates with the test sample. After a suitable period of incubation in a maintenance medium, the presence of cysts or oocysts is determined by direct microscopy, IFA staining, detection of protozoan-specific nucleic acids, or ELISA.

Advantages. This method specifically measures cysts and oocysts that are infectious to human cells, thereby eliminating false positive counts from non-infectious cysts and oocysts and from species that are not of concern for human health. ELISA-based scoring of infected cultures should facilitate the automated determination of Most Probable Numbers of protozoa in samples.

Limitations. The method is subject to all of the limitations of concentrating samples by filtration. In addition, it is slower because of the incubation period required to propagate protozoa in the infected cells, and the cell cultures are subject to cytotoxic effects from other contaminants in the samples. The method may not

reveal sources of protozoan contamination because the sample may contain cysts or oocysts that have been inactivated. Specialized equipment and techniques are required to prepare and maintain cell cultures for the assay. No formal determination of method detection limit has been done. Not throughly tested with environmental water matrices.

Enzyme-Linked Immunosorbent Assay (ELISA)

Summary. This method measures the activity of an indicator enzyme attached to antibody molecules that have bound to cysts or oocysts (De La Cruz and Sivaganesan 1994, Woods et al. 1995). The amount of enzyme activity is presumed to be related to the concentration of cysts or oocysts in the water sample. A sample concentrated by filtration or other methods is reacted with antibody specific for cysts or oocysts which is then coupled to an enzyme. After unbound enzyme is washed away, activity of the remaining enzyme is measured as development of a characteristic color when a chromogenic substrate is added. The quantity of bound enzyme is estimated by comparing the intensity of color with a standard curve made using a known number of cysts or oocysts.

Advantages. Technically easy and inexpensive to perform. Microscopy is not required, eliminating the need for an expensive microscope and a highly trained microscopist. Kits are available to facilitate the ELISA analysis, and ELISA methods can be automated.

Limitations. Limitations of the concentration techniques apply. The limit of detection of for samples in reagent water is about 10 oocysts per aliquot analyzed (10 to 50 μg of pellet), much higher than that of the ICR method. However, no formal determination of method detection limit has been done. Not throughly tested with environmental water matrices. Because the secondary antibody reacts with antibody molecules rather than cyst or oocyst structures, the color reaction is less specific, and the method is less amenable to simultaneous identification of cysts and oocysts. Excess turbidity can mask color development, further reducing sensitivity. Cross-reactivity or non-specific adsorption of antibody (e.g., to algal cells) and adsorption of antibody to cyst or oocyst fragments can cause false positive results. The method does not indicate whether cysts or oocysts are viable.

Polymerase Chain Reaction Method (PCR)

Summary. This method uses specific DNA primers and DNA polymerase to amplify *Giardia* (Abbaszadegan et al. 1991) and *Cryptosporidium* (Johnson et al. 1995) gene sequences. Extracts of cysts and oocysts purified by filtration and/or centrifugation or foci of infection in cell cultures (Rochelle et al. 1997a) are reacted with specific DNA primers and DNA polymerase through several cycles of DNA sequence amplification (as much as a 10^6-fold amplification of the target DNA molecule). The quantity of DNA synthesized is measured and its genetic identity can be confirmed by hybridization with known *Giardia* (Abbaszadegan et al. 1991, Awad-el-Kariem et al. 1994) or *Cryptosporidium* (Johnson et al. 1995) DNA.

Advantages. The method is rapid and does not require specialized microscopy, and the reagents other than primers are available in kit form at relatively low cost for routine use. It is possible to identify species of

Giardia and *Cryptosporidium* by careful choice of primer sequences (Rochelle et al. 1997b). Modifications of the technique may lead to automated detection and identification of protozoa (Paszko-Kolva et al. 1995) and may make it possible to identify viable oocysts (Abbaszadegan et al. 1997a, Rochelle et al. 1995, Wagner-Wiening and Kimmig 1995).

Limitations. Limitations of cyst and oocyst concentration techniques apply. Only a small aliquot (<100 µL) of concentrated sample can be analyzed. In addition, the method is sensitive to organic materials, especially humic and fulvic materials, and other debris that may be concentrated with the cysts and oocysts. Techniques for purifying cysts and oocysts need further development (e.g., purification by antibodies bound to magnetic beads). The method may be able to detect a single cyst or oocyst per sample (Stinear et al. 1996), although the method detection limit has not been formally determined. Not throughly tested with environmental water matrices.

Identification of Ribonucleic Acid Sequences by Confocal Laser Scanning Microscopy

Summary. This method uses in situ hybridization of a ribosomal ribonucleic acid (rRNA) probe to identify species of parasites. Oligodeoxyribonucleotides copied from cloned *Cryptosporidium* rRNA sequences (Cai et al. 1992) are labeled with fluorescent dye and hybridized with *Cryptosporidium* rRNA in environmental samples that have been concentrated by filtration and/or centrifugation. To enhance resolution, fluorescence is measured by confocal laser scanning microscopy (Caldwell et al. 1992).

Advantages. This method allows *Cryptosporidium* oocysts pathogenic to humans to be distinguished from species that are not pathogenic to humans. It does not require microscopic identification of characteristic morphology, and it may allow the identification of viable oocysts.

Limitations. Limitations of oocyst concentration techniques apply, and non-viable oocysts may not be detected, preventing the identification of potential environmental sources of contamination. This method requires highly specialized equipment and is still under development. It does not appear to be appropriate for routine analytical use. No formal determination of method detection limit has been done. Not throughly tested with environmental water matrices.