

A SURVEY OF SOURCE AND FINISHED WATER SUPPLIES FOR  
GIARDIA CYSTS AND CRYPTOSPORIDIUM OOCYSTS

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In Partial Fulfillment  
of the Requirements for the Degree  
Master of Science in Biology

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by

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## ABSTRACT

# A SURVEY OF SOURCE AND FINISHED WATER SUPPLIES FOR *GIARDIA* CYSTS AND *CRYPTOSPORIDIUM* OOCYSTS

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The occurrence of *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts was determined from source and treated drinking water supplies at selected water treatment facilities in Eastern Kentucky. *Giardia* and *Cryptosporidium* are two human protozoan parasites often found in water supplies due to fecal contamination. Characteristic cysts and oocysts were identified from large volume water samples, typically 100 gallons for raw samples and 1,000 gallons for finished water, using an indirect fluorescent antibody specific for *Giardia* and *Cryptosporidium*. Source water from four different water treatment facilities utilizing a reservoir, river, and two creeks were assayed. All sites exhibited *Giardia lamblia* cysts while *Cryptosporidium parvum* oocysts were detected at all sites except those collected from the reservoir for a 100% and 75% occurrence rate for these two protozoans,

respectively. Samples from two of the four (50%) water treatment facilities surveyed yielded *Cryptosporidium* oocysts in finished water whereas no *Giardia* cysts were detected utilizing this method.

Water quality indicators, including pH, turbidity, and fecal coliform bacteria levels were taken on all raw water samples in an effort to predict *Giardia* and *Cryptosporidium* contamination in water sources. Statistical analysis of specified indicators determined that neither pH, turbidity, nor fecal coliform bacteria exhibited significant correlations with detected cysts or oocyst levels.

The treatment train of each water treatment facility was surveyed to determine if *Giardia* and *Cryptosporidium* levels were influenced by physical/chemical parameters. Of the three filtration methods utilized: 1) direct; 2) slow sand; and 3) rapid sand filtration, none were shown to be significantly different from each other in affecting protozoa levels. Disinfection and purification of raw water sources varied at each facility surveyed. An analysis of chemical additions and chlorine contact times also proved to be insignificant in their affects on detected *Giardia* cysts and *Cryptosporidium* oocysts levels. No oocysts were detected in treated water in plants using a polymer (cat-floc-tl) as a coagulant; however, those that utilized alum as a coagulant, *Cryptosporidium* oocysts were detected in their finished water.

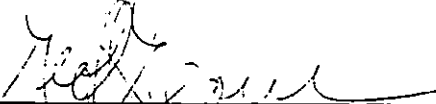
*Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts were demonstrated to be present in source water utilized by four water treatment plants in Eastern Kentucky. Of the standard water quality indicators often utilized for raw water sources, none were shown to be

reliable indicators in alerting water treatment facilities of possible *Giardia* or *Cryptosporidium* contamination. Nonetheless, *Cryptosporidium* oocysts were detected in two of four treated drinking water supplies. This study has demonstrated that current filtering and disinfection techniques need to be improved, along with best management practices in the watersheds, to diminish the threat of infection by *Giardia lamblia* and *Cryptosporidium parvum* by drinking treated water, especially in immunosuppressed individuals.

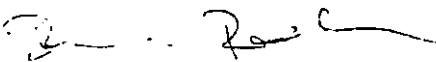
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## Chapter 1 Introduction

### Significance

Two parasitic protozoa presently threatening the safety of drinking water supplies nationwide are *Giardia lamblia* and *Cryptosporidium parvum*. Upon ingestion, these two organisms lead to many gastrointestinal symptoms including gas, bloating, intermittent diarrhea and constipation, abdominal cramps, nausea, fever, and weight loss (Hunter, 1993). Several waterborne outbreaks have been attributed to each protozoan.

*Giardia* is the most frequently identified etiologic agent in waterborne outbreaks. Over the past 25 years, 106 outbreaks of waterborne giardiasis have been reported in the United States (Rose et al., 1991). Additionally, between 1991 and 1992, four more outbreaks associated with drinking water occurred in California, Nevada, Idaho, and Pennsylvania (Moore et al., 1994). The first documented waterborne outbreak of giardiasis in the United States occurred in Aspen, Colorado in 1965 (Craun, 1979). An outbreak of protracted, intermittent diarrhea occurred in persons inhabiting a ski lodge in Aspen. A survey of 1,094 skiers revealed illness developed in at least 11.3 %. Fifty-nine persons were found to be excreting *Giardia lamblia* cysts in their stool. Environmental studies determined contaminated well water by sewage leaking from defective lines near the wells. *Giardia lamblia* cysts were found in the broken pipes causing the waterborne spread of giardiasis (Moore et al., 1969).

*Cryptosporidium* is also becoming a common agent of waterborne outbreaks of disease. First described in 1976, cryptosporidiosis is normally self-limiting in immunocompetent individuals but can prove to be life-threatening in immunodeficient individuals, e.g. HIV positive individuals, the elderly, and those undergoing chemotherapy (Current et al., 1983; Wolfson et al., 1985). Waterborne outbreaks of cryptosporidiosis are widespread, occurring in such places as Albuquerque, New Mexico (Gallaher et al., 1989), and Carrolton, Georgia (Hayes et al., 1989), and most recently in Milwaukee, Wisconsin (Nash, 1993). The Milwaukee cryptosporidiosis outbreak occurred in late March and early April, 1993. The outbreak affected over 400,000 people causing symptoms of profuse diarrhea, abdominal pain, nausea, vomiting and fever. To date, 100 deaths have been reported in Milwaukee as a result of the cryptosporidiosis outbreak. Prior outbreaks of cryptosporidiosis affected 13,000 people in Carrolton, Georgia in 1987 (Hayes et al., 1989), 68 people in New Mexico between 1984-1986 (Gallaher et al., 1989), and 43 people in Jackson County, Oregon in 1992 (Leland et al., 1993). Currently there is no cure for cryptosporidiosis.

To address the increasing numbers of *Giardia* and enteric viral waterborne outbreaks, the United States Environmental Protection Agency (USEPA) enacted the Surface Water Treatment Rule on June 29, 1989 requiring all surface water to be filtered and disinfected to control enteric protozoa and viruses. The rule set forth several criteria:

1. Systems would be required to ensure overall removal and or inactivation of at least 99.9% of *Giardia* cysts;
2. Systems would be required to continuously monitor disinfection residuals and ensure at least a 0.2 mg/L disinfectant level would enter the system at all times;
3. It would be expected that the disinfection residual would supplement the filtration process by achieving at least a 0.5 log<sub>10</sub> inactivation factor; and
4. Systems would be required to ensure that filtered water turbidities be < 0.5 NTU (Nephelometric Turbidity Unit) in 95% of the measurements taken every month (LeChevallier, 1991).

The rule establishes criteria that all water plants must follow concerning *Giardia* and enteric viruses detection limits. However, no criteria has been set forth for *Cryptosporidium* detection and very little data is available to determine optimum water treatment practices for the organism.

### **Need for the Study**

This study was undertaken to determine if *Giardia* cysts and *Cryptosporidium* oocysts are present in raw and treated water processed by selected water treatment plants in Eastern Kentucky. LeChevallier et al. (1991) reported that treatment plants with high levels of cysts and oocysts in raw water supplies were more likely to have *Giardia* cysts and *Cryptosporidium* oocysts detected in their finished drinking water. Little data is currently available to suggest if Kentucky surface waters

contain levels of *Giardia* or *Cryptosporidium* that might pose a consumer health hazard. Additionally, the study will attempt to demonstrate a correlation between the presence of high turbidity, fecal coliform, and/or pH with the presence of *Giardia* or *Cryptosporidium* in water sources. This study may be used by water treatment facilities to better understand the correlation between physical/biological indicators and the presence of *Giardia* or *Cryptosporidium* in both source and treated drinking water.



## Objectives of the Study

1. To determine the prevalence of *Giardia* cysts and *Cryptosporidium* oocysts in raw water sources utilized by four Eastern Kentucky water treatment plants.
2. To determine the prevalence of *Giardia* cysts and *Cryptosporidium* oocysts in filtered drinking water in four Eastern Kentucky water treatment plants.
3. To evaluate the efficiency of selected water plants in removing *Giardia* cysts and *Cryptosporidium* oocysts from drinking water supplies.
4. To determine whether turbidity levels can serve as an indicator to the presence of *Giardia* cysts and *Cryptosporidium* oocysts.
5. To determine if the pH of treated and raw water sources correlate with levels of *Giardia* cysts and *Cryptosporidium* oocysts.
6. To determine whether fecal coliforms can serve as an indicator for the presence of *Giardia* cysts and *Cryptosporidium* oocysts.

## Chapter II Literature Review

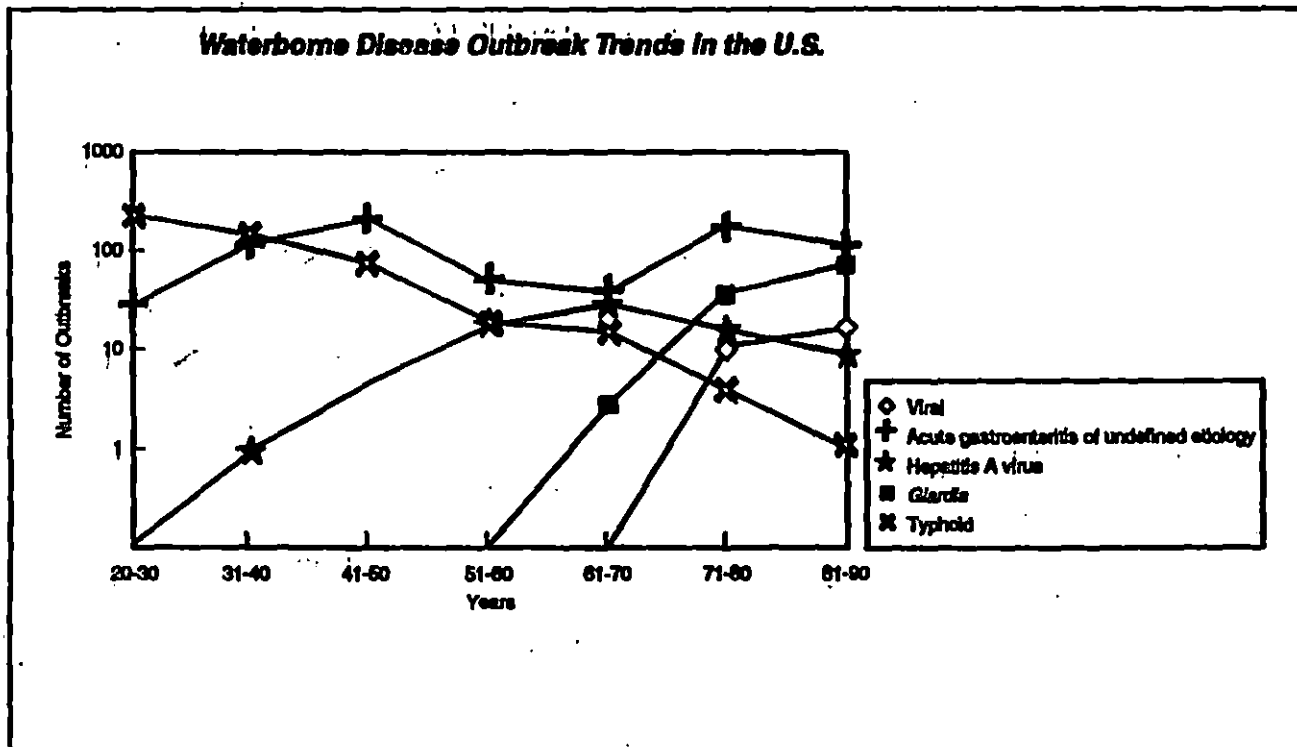
Surface drinking water supplies have long been known to harbor many pathogenic microbial agents. Current strategies for controlling these microbes are based on regulations that specify treatment qualities of the source water along with acceptable limits to water quality indicators such as coliform bacteria, turbidity, and heterotrophic plate count bacteria (Logsdon, 1987). These strategies have historically been fairly successful in reducing epidemic and endemic waterborne bacterial diseases.

Overall, the strategies devised early in the twentieth century to control waterborne microbial disease, such as chlorination and filtration, have changed very little. However, waterborne diseases are now known to be caused by a much broader variety of microbes than the enteric bacteria which the control measures were designed to eliminate (Figures 1-4) (Sobsey et al., 1993). Among the growing list of heretofore unstudied agents of waterborne disease are two parasitic protozoans: *Giardia lamblia* and *Cryptosporidium parvum*.

### **Giardia lamblia Background**

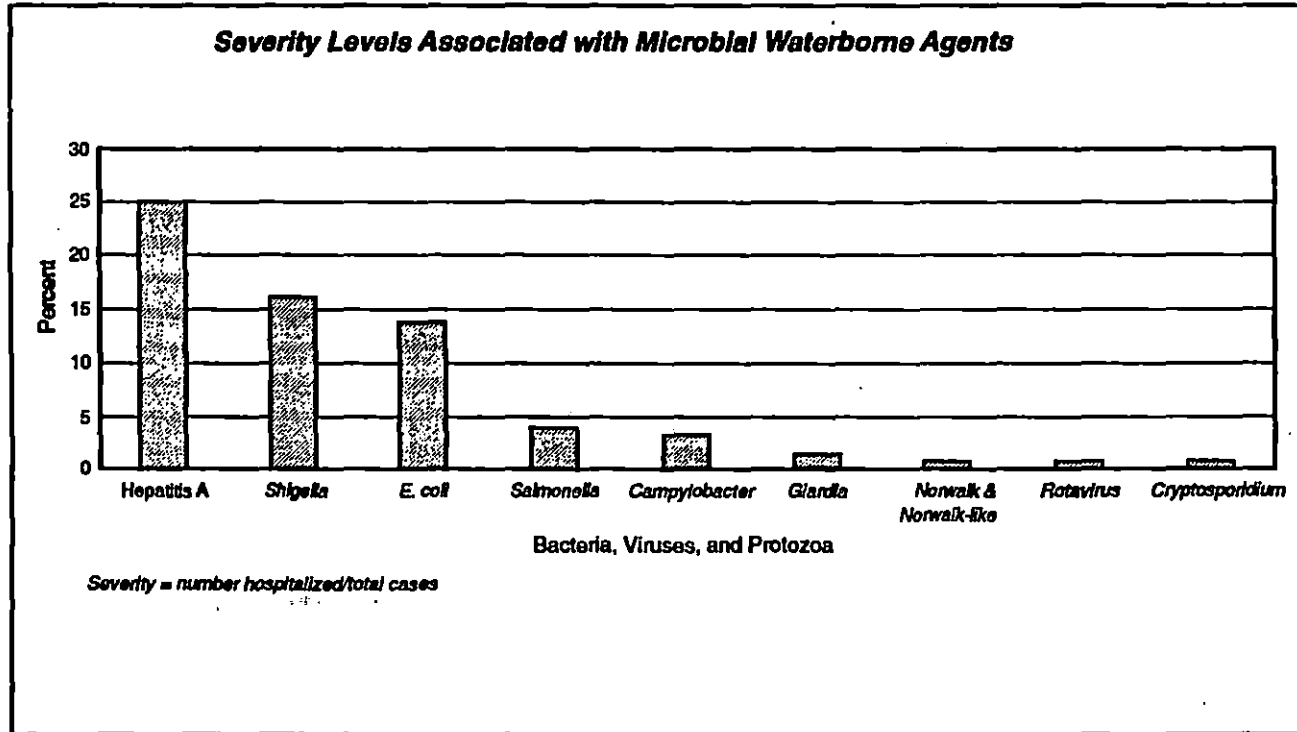
*Giardia lamblia*, the most frequently isolated enteric protozoan (Sauch, 1984; Sauch, 1985; Rose et al., 1991), is an intestinal parasite often transmitted in drinking water during the cyst phase of its life cycle (Hunter, 1993). Species of *Giardia* are not host specific and have been found in humans, beavers, dogs, cats, muskrats, rodents, cattle, and

Figure 1 Waterborne Disease Outbreaks—Trends in the U.S.



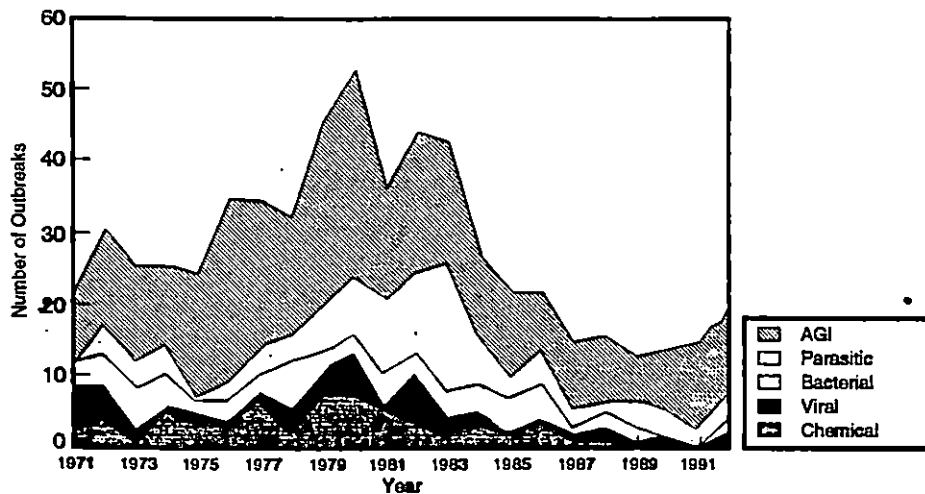
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Figure 2 Severity Levels Associated with Microbial Waterborne Agents



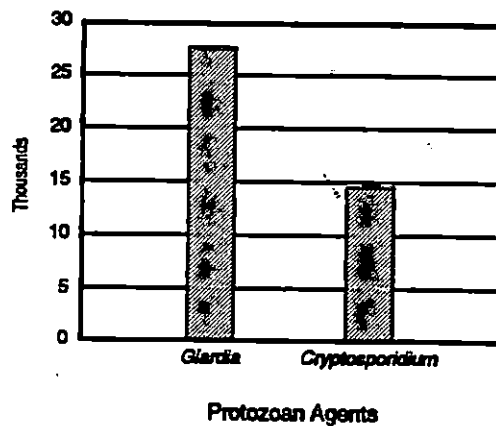
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Figure 3 Waterborne Outbreaks, by Year and Etiologic Agent — United States, 1971-1992



AGI - acute gastrointestinal illness of unknown etiology

Figure 4 Cases of Documented Protozoan Waterborne Disease in the U.S., 1971-1990



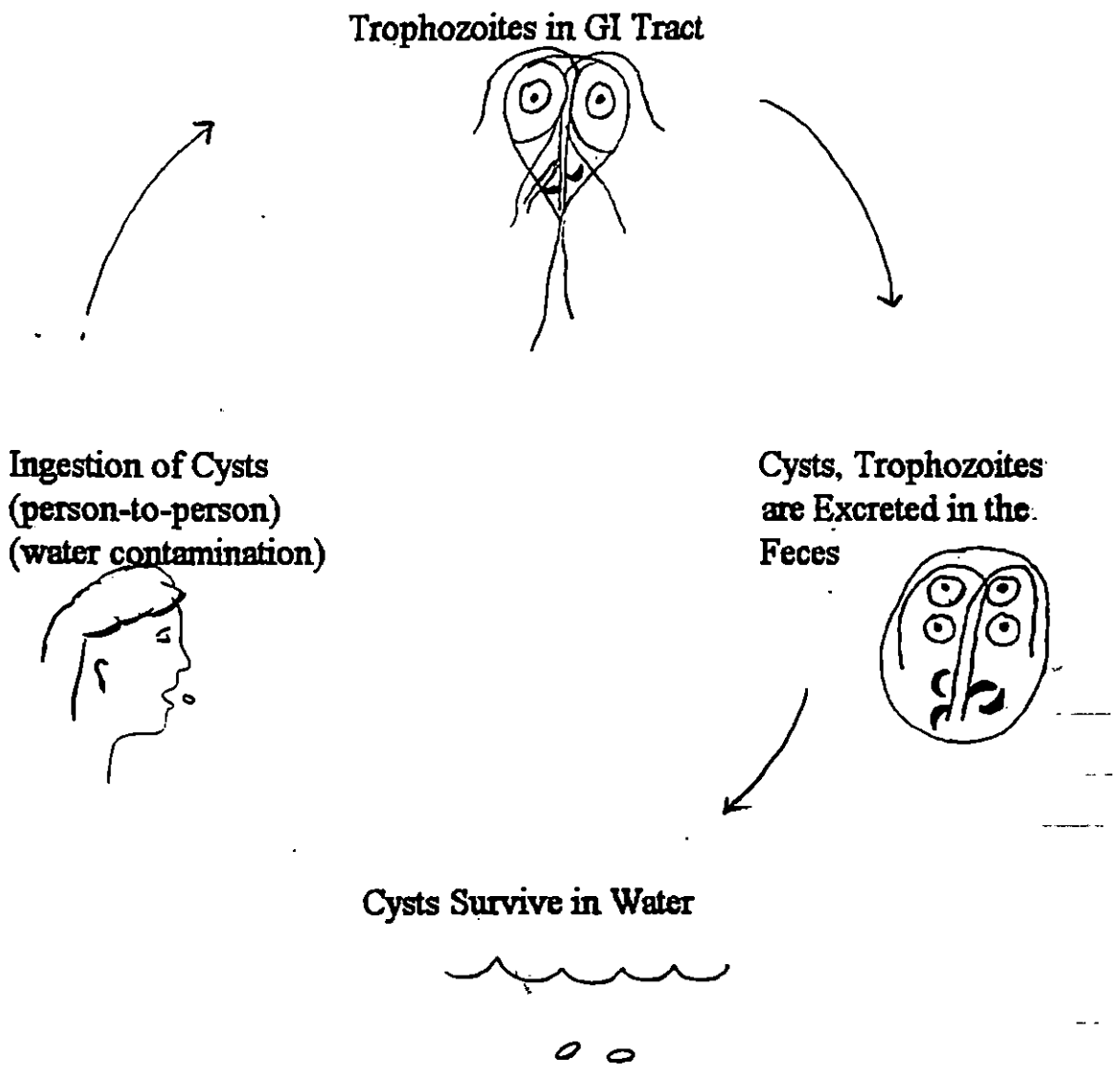
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sheep (McFeters, 1990). *Giardia lamblia* lives in the duodenum, jejunum, and upper ileum of humans with an adhesive disc fitting over the surface of epithelial cells. The life cycle consists of two stages: the active feeding trophozoite and an environmentally resistant cyst (Figure 5). Only the trophozoites are found in the small intestine but the parasite can encyst and leave the host's body in the feces (Schmidt and Roberts, 1989). The cysts are the infective stage of the *Giardia* life cycle and may contaminate water sources. *Giardia* cysts are environmentally stable and resistant to inactivation by normally used drinking water disinfectants (Rose et al., 1991).

Source water contamination occurs mainly when environmentally stable cysts are excreted in the feces of infected animals and humans. Infected feces then enters water supplies via water run-off enabling a fecal-oral route of transmission (Jarroll et al., 1981; Roes et al., 1988). In an addition to larger epidemics, lower levels of *Giardia* cysts may periodically enter water supplies, leading to ongoing low levels of giardiasis that go undetected. Such low cyst levels are more difficult to detect but may still pose a serious risk to public health, particularly in immunosuppressed individuals (Glicker and Edwards, 1991).

Giardiasis outbreaks have been reported worldwide. Most identified cases of waterborne giardiasis in the United States have been epidemic. Waterborne giardiasis was first recognized in the United States in 1965, and as of 1988, 106 outbreaks had been reported (Rose et al., 1991). From 1989-1990, 16 states reported 26 additional outbreaks of disease due to contaminated drinking water resulting in an

Figure 5  
*Giardia lamblia* Life Cycle



estimated total of 4,288 illnesses (Herwaldt, 1991). Of the 12 outbreaks in which the agent was identified, *Giardia lamblia* was responsible for seven. The source of the ingested *Giardia* cysts was unfiltered surface water or surface-influenced groundwater (Herwaldt, 1991). In 1991 and 1992, 17 states and territories reported 34 outbreaks of disease associated with drinking water affecting 17,464 people. *Giardia lamblia* was the cause of four (12%) outbreaks in which the causative agent was determined (Moore et al., 1994).

### **Giardiasis Case Study: Pittsfield, Maine**

In the period of November 1, 1985 to January 31, 1986, 703 cases of giardiasis were reported in Pittsfield, Massachusetts. The city obtained its water supply from two main reservoirs (A&B) and an auxiliary reservoir (C). The drinking water from the sites was chlorinated at the treatment plant but unfiltered. While alterations were being made at the water treatment facility to install a filtration system, the reserve C reservoir was relied on heavily as the major water source for the town. Illness peaked approximately two weeks after the city began pumping from reservoir C. The attack rate of giardiasis for residents of areas supplied by reservoir C was 14.3 per 1,000 persons, compared with 7.0 per 1,000 persons in areas not receiving water from reservoir C. Environmental studies discovered *Giardia lamblia* in reservoir C. Cysts were found in reservoirs A & B, but at much lower concentrations than C. This case demonstrated the importance of



filtration coupled with chlorination as a way to reduce the risk of waterborne giardiasis (Kent et al., 1988).

### **Disinfection Procedures for *Giardia lamblia***

A great concern for public health now exists because *Giardia* cysts are extremely resistant to normal water treatment processes. Chlorination standards are 0.5 mg chlorine/L treated water (Lin, 1985); however, *Giardia* cysts may require higher chlorine concentrations and longer contact times to be inactivated, especially in treatment systems that lack filtration. For example, Jarroll et al. (1981) tested *Giardia* cyst viability at different water temperatures, pHs, chlorine concentrations, and chlorine contact times (Table 1). Longer chlorine contact times and higher chlorine concentrations (up to 2 mg/L) were needed to destroy *Giardia lamblia* cysts at low water temperatures. Further, disinfection by chlorine was most effective at lower pH levels (6 and 7). The study emphasized that when chlorine is used as a cysticide against *Giardia lamblia*, treatment systems should monitor pH, chlorine demand, and temperature of the water being treated (Jarroll et al., 1981). Unfortunately, many water treatment facilities do not follow these guidelines.

To prevent waterborne transmission of *Giardia* and other infectious agents, the United States Environmental Protection Agency (USEPA) has prepared criteria for filtration and disinfection of all public water systems using surface water sources. The criteria are included in the Surface Water Treatment Rule (SWTR) passed

Table 1      Summary of Chlorine Effects on *Giardia* Cyst Viability as Reported by Jarroll et al., 1981

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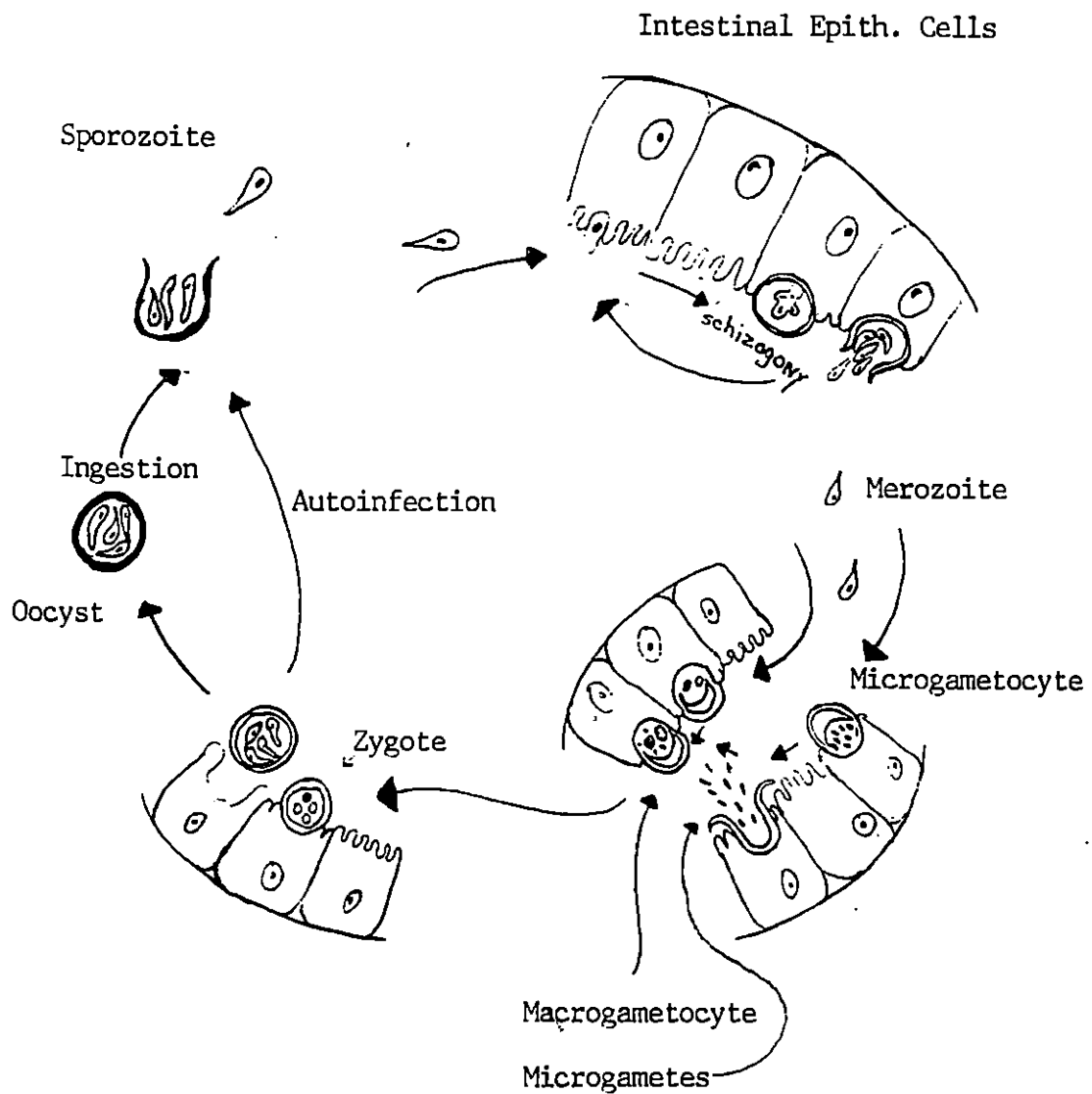
1. 1.5 mg chlorine/Liter water killed all cysts at 25 °C at pH 6, 7, and 8.
  2. 2.5 mg chlorine/Liter water killed all cysts at 15 °C at pH 6, but at pH 7 and 8 small numbers of cysts remained viable after 30 minutes but not after 60 minutes.
  3. 1.0 mg chlorine/Liter water failed to kill all the cysts at 5 °C at all pHs tested after 60 minutes exposure.
  4. 2.0 mg chlorine/Liter water killed all cysts at 5 °C pH 6 and 7 after 60 minutes but not at pH 8.
  5. 4.0 mg chlorine/Liter water killed all cysts at all three pH values after 60 minutes but not after 30 minutes.
  6. 8.0 mg chlorine/Liter water killed all cysts at pH 6 and 7 after contact for 10 minutes and at pH 8 after 30 minutes.
- 

\*Jarroll, Bingham, and Meyer , 1981

June 29, 1989 (LeChevallier et al., 1991). The intent of the Surface Water Treatment Rule was to reduce the risk of acquiring a waterborne infection of *Giardia* spp. to below an annual rate of  $10^{-4}$  per person. One case of microorganismal caused illness per year per 10,000 people is the goal. To achieve  $10^{-4}$  annual risk of *Giardia* infection, it has been calculated that potable water should not contain more than  $7 \times 10^{-4}$  *Giardia* cysts per 100 liters water on the basis of the geometric mean for one year. Therefore, treatment plants assume that *Giardia* levels in raw water are not greater than 7 cysts per 100 liters (LeChevallier et al., 1991).

#### **Cryptosporidium parvum Background**

In recent years, *Cryptosporidium parvum* has become increasingly prevalent as an agent of waterborne disease, especially in immunodeficient persons (Current et al., 1983; Wolfson et al., 1985). Although known since 1907 (Jokipii et al., 1985), *Cryptosporidium parvum* was first recognized as an agent of waterborne disease in 1987. *Cryptosporidium* oocysts have been isolated from such animals as cat, cattle, humans, dog, goat, mouse, pig, rat, and sheep (McFeters, 1990). *Cryptosporidium* species have a complex life cycle which includes infection of the brush border of epithelial cells in the human intestinal tract (Figure 6). *Cryptosporidium* form environmentally stable spherically shaped oocysts that are traditionally 2 to 5  $\mu\text{m}$  in diameter (Levine, 1984; Ongerth and Stibbs, 1987). This protozoan has a cosmopolitan distribution.

Figure 6 Cryptosporidium parvum Life Cycle

### **Cryptosporidiosis Outbreaks**

Despite its fairly recent classification as a waterborne pathogen, *Cryptosporidium* has been associated with several disease outbreaks (Figure 7). In mid-January, 1987, a cryptosporidiosis outbreak was reported in Carrollton, Georgia. Between January 12 and February 7, 1987, an outbreak of gastroenteritis affected an estimated 13,000 people in a county of 64,900 residents. An investigation of state and federal epidemiologists indicated the outbreak involved the entire county. *Cryptosporidium* oocysts were identified in 58 of 147 (39%) patients tested during the outbreak. Even though the sand-filtered and chlorinated water system met all regulatory-agency quality standards, *Cryptosporidium* oocysts were found in the treated water. Further investigations determined that sub-optimal flocculation and filtration probably allowed parasites to pass into the drinking water supply (Hayes et al., 1989).

From January to June, 1992, a large outbreak of cryptosporidiosis occurred in Jackson County, Oregon. Epidemiological investigations associated 43 cases of cryptosporidiosis with the drinking water system in the small community of Talent. Municipal water systems primarily used rapid filtration treatments. Mechanical and operational deficiencies at one of the city's water filtration plants, along with unusually poor raw water supply conditions, were possible causes for the outbreak (Leland et al., 1993).

More recently an outbreak of cryptosporidiosis affected over 400,000 Milwaukee citizens. On April 5, 1993, local health officials

**Figure 7 Cryptosporidiosis Outbreaks in North America and U.K.**

<b>Date</b>	<b>Location</b>	<b>Approximate Numbers of Cases</b>	<b>Source/Treatment</b>
1984	Braun Station San Antonio, Texas	2,000	Well/Chlorinated
1986	Albuquerque, New Mexico	56	Lake/Untreated
1987	Carrollton, Georgia	13,000	River/Conventional
1989	Swindon/Oxfordshire, U.K.	55,000	Reservoir/Conventional
1992	Jackson County, Oregon	3,000	Spring/Chlorinated River/Package Filtration
1992	Pennsylvania	551	Well
1993	Milwaukee, Wisconsin	400,000	Lake/Conventional
1993	Waterloo/Kitchener, Ontario, Canada	1,000	River/Conventional Preozonation

\*\*AWWA Teleconference

were alerted to the outbreak as suffering residents began calling the city health department. The origin of the *Cryptosporidium* contamination of the drinking water still remains a puzzle. One possibility is that contaminated runoff from a farm could have made its way into the plant source water, Lake Michigan (Nash, 1993). However, the plant never violated state and federal drinking water regulations.

### **Disinfection Procedures for *Cryptosporidium parvum***

*Cryptosporidium* may be found in surface waters in all regions of the United States, often predominating in mountainous areas (LeChevallier et al., 1990). *Cryptosporidium* oocysts are extremely resistant to most commonly used disinfectants. Recent studies by Korich et al. (1990) demonstrated that oocysts viability was not affected by exposure to 1.05 and 3.0% chlorine as sodium hypochlorite for up to 18 hours. Long term exposure to 10% formalin, 5-10% ammonia, and 70-100% bleach was deemed necessary to completely eliminate oocyst infectivity. This drastic technique can be replaced by treatment with 1.11 ppm of ozone which completely eliminates infectivity in 5 minutes.

### **Infectious Dosage of *Cryptosporidium* and *Giardia***

An exact number of *Cryptosporidium* oocysts that must be ingested to cause infection in humans is not known. Studies to date indicate that as few as 10 and perhaps as many as 500 oocysts are required to initiate infections in mammals. The infective dose for humans is thought to be fewer than 10 oocysts. Additional studies are

being conducted by the EPA to verify these findings (Pontius, 1994). When determining an infectious dose, a person's immune status must be taken into consideration (Current and Garcia, 1991). In patients with immune deficiencies, such as AIDS patients, length and severity of illness may depend on the ability to reverse immunosuppression. Thus, an infectious *Cryptosporidium* dosage for one person may differ greatly from that seen in another.

Similarly, a definitive infective dosage of *Giardia* is unknown. *Giardia* spp./strains have been shown to demonstrate low infectious dose, with as few as 10 cysts capable of initiating infection (Rose et al., 1991). Levels of cysts ranging from 0.6 to 21/100 L have been associated with waterborne giardiasis outbreaks (Rose et al., 1991). Giardiasis infections have been shown to last as short as four weeks and as long as ten years. An infective dosage may depend upon the virulence of individual strains of *Giardia* or/and with host immune factors (Jakubowski et al., 1985).

### **Water Treatment Practices to Prevent Waterborne Disease**

Water treatment practices have played a major role in the prevention of waterborne disease. Traditional treatments such as coagulation, sedimentation, filtration, and disinfection have been shown to remove 99.9% of *Giardia* cysts (DeWalle, 1984). Other effective *Giardia* treatment processes such as rapid sand filtration (Al-Ani et al., 1986), diatomaceous earth (Lange et al., 1986), and slow sand filtration (Bellamy et al., 1986; Fogel et al., 1993) are also effective (Logsdon,



1990). Current literature does not specify treatment practices for *Cryptosporidium*. Several factors make *Cryptosporidium* oocysts more difficult to treat, such as: 1) smaller size, 2) lower sedimentation rates, and 3) increased resistance to disinfection (LeChevallier et al., 1991).

Upon finalizing the USEPA Surface Water Treatment Rule, several criteria were established for systems that filter and disinfect. Exactly how these criteria will affect the quality of treated drinking water is unclear. For example, filter plants are required to meet a 0.5 NTU turbidity limit; however, Logsdon et al. (1990) showed that *Giardia* cysts could pass through treatment filters with relatively small changes (0.2 to 0.3 NTU) in turbidity levels. Also, the concentration of parasites in source waters is unknown, and there are currently no raw water indicators of parasite densities (LeChevallier and Norton, 1992).

Current literature recognizes no filter performance criteria for *Cryptosporidium parvum*. As a result, various studies have detected *Cryptosporidium* in treated water supplies. For example, Madore et al. (1987) found  $1.30 \times 10^3$  *Cryptosporidium* oocysts per liter of filtered sewage effluents with highly variable oocyst numbers found in surface waters. Rose (1988) detected *Cryptosporidium* oocysts in 20% (two of ten) of the filtered water supplies tested in the western United States.

In a recent study to determine whether or not compliance to the SWTR would ensure control of protozoans in potable water supplies, *Giardia* and *Cryptosporidium* levels were tested in source and effluent water of 66 water treatment plants in 14 states and 1 Canadian province. The results showed that cysts and oocysts were widely dispersed in the

aquatic environment. *Giardia* spp. were detected in 81% of the raw water samples while *Cryptosporidium* spp. were found in 87% of the raw water samples (LeChevallier et al., 1991). More disturbing was that *Giardia* cysts were detected in 17% of the 83 filtered water effluents and *Cryptosporidium* oocysts were observed in 27% of the drinking water samples. Overall, cysts or oocysts were found in 39% of the treated effluents. Compliance with the filtration criteria outlined by the SWTR did not ensure that treated water was free of cysts and oocysts (LeChevallier et al., 1991).

### **Water Quality Indicators for *Giardia* and *Cryptosporidium***

Investigators have tried to correlate *Giardia* and *Cryptosporidium* levels in surface waters with various water quality indicators. For example, LeChevallier and Norton (1992) examined turbidity and its usefulness in predicting *Giardia* and *Cryptosporidium* densities (Table 2). Three test sites were used that had varying turbidity: low (site 3), moderate (site 2), and high (site 1). Results showed *Giardia* and *Cryptosporidium* were detected in all raw-water samples from the high turbidity plants. Parasites were detected in nine of ten raw water samples of the moderate turbidity plant while *Giardia* and *Cryptosporidium* parasites showed up in eight of ten raw-water samples at the low turbidity plant. The authors found a significant correlation between parasite densities and turbidity levels in source waters (LeChevallier and Norton, 1992). In a study by Al-Ani et al. (1986), it

Table 2 Turbidity and Parasite Correlations In Raw Water  
Demonstrated by LeChevallier and Norton, 1992

Site	Average Turbidity	Prevalence in Samples	Distribution Range
1	<5 to >240 NTU	<i>Giardia</i> 5 of 10 <i>Crypto.</i> 10 of 10	1.13-31.1/L .82-71.9/L
2	<2 to >75 NTU	<i>Giardia</i> 8 of 10 <i>Crypto.</i> 7 of 10	.14-64.2/L .42-5.1/L
3	<.3 to 5 NTU	<i>Giardia</i> 2 of 10 <i>Crypto.</i> 7 of 10	2.85/L .77-8.7/L

\*For all three sites combined, *Giardia* levels correlated ( $p < 0.01$ ) with fecal coliform, total coliform, HPC bacteria, turbidity, pH, and particle counts.

\*Overall, raw water *Cryptosporidium* levels correlated with only total coliform bacteria at  $p < 0.01$  and with HPC bacteria, turbidity, and particle counts at  $p < 0.05$ .

was demonstrated that when effective chemical pretreatment was employed in combination with rapid rate filtration, *Giardia* cysts and turbidity levels were reduced from 80 to 99%. It was also observed that for waters having turbidities <1 NTU, percent reduction of turbidity levels served as an indicator of the removal of *Giardia* cysts.

Logsdon et al. (1985) performed a pilot study to evaluate the efficiency of sedimentation and various filter media in the removal of *Giardia* cysts. Turbidity was also measured. Removal of cysts by sedimentation ranged from 65 to 93%. Removal correlated with turbidity levels; turbidity changes as small as 0.2 to 0.3 NTU were associated with the increase in cyst concentration.

It should be noted that not all researchers have demonstrated that turbidity levels can serve as parasite indicators. Rose et al. (1988) examined watersheds in the western United States for *Giardia* and *Cryptosporidium*. From 39 samples, *Cryptosporidium* and *Giardia* were detected in 20 and 12 of the samples, respectively. Turbidity was shown not to be a reliable predictor of the enteric protozoa.

In November, 1981, a giardiasis outbreak occurred within the Highlands Water and Sanitation district in Colorado. Investigators showed that the initial cause of the outbreak was a reduced chlorine contact time even though the plant had numerous design and operational deficiencies such as lack of chemical treatment, improper backwashing, and poor application of raw water to filters. Despite these conditions, the turbidity level remained within acceptable ranges. Turbidity levels

did not indicate the increased level of *Giardia* cysts (Braidech and Karlin, 1985).

Current literature also shows conflicting evidence as to whether coliform bacteria levels can be used as indicators for *Cryptosporidium* oocysts and *Giardia* cysts. LeChevallier and Norton (1992) reported that both total and fecal coliform levels showed significant correlations with *Giardia* and *Cryptosporidium* levels in raw water sources. Conversely, Rose et al. (1988) reported that neither total or fecal coliform levels were reliable indicators of *Giardia* and/or *Cryptosporidium* contamination. Rose et al. (1991) tested *Cryptosporidium* and *Giardia* levels in 257 samples in 17 states across the United States. Their study found no association between the protozoa and bacterial indicators. The authors concluded that other indicators need to be established to enable determination of the potential risk of enteric protozoan contamination.

Payment and Franco (1993) suggest any organism used as a water quality indicator must withstand several criteria: 1) the microorganism should be present at the same time as the target pathogen, 2) it should be quantitatively at least as abundant, 3) it should be at least as resistant to the treatments and survive slightly better, and 4) it should be easy to enumerate by methods that are specified and sensitive. Past studies have demonstrated that coliforms are inadequate to indicate the presence of pathogens, especially parasites. Thus, current research seems to focus on anaerobic bacteria, such as *Clostridium perfringens*. Payment and Franco (1993) collected water

samples from three water treatment plants, testing for the presence of pathogenic protozoa. Several water quality indicators were taken and it was demonstrated that only *Clostridium perfringens* counts correlated with *Giardia* and *Cryptosporidium* densities, suggesting it may be a suitable indicator for the presence of pathogenic protozoa.

In a separate study, water samples were collected from wastewater, sludge, and natural water bodies, such as rivers and lakes. *Clostridium perfringens* chlorine resistance was calculated as 99% inactivation in 0.25 minutes at 1.0 mg/L chlorine. Because *Clostridium perfringens* is more resistant to chlorine than other microorganisms, it could be a reliable indicator for fecal contamination (Hirata et al., 1991).

### **Detection of *Giardia* and *Cryptosporidium***

Besides using water quality indicators as a proxy, many investigators have proposed modified methods for direct detection of *Giardia*. Much research has been done to determine which methods and materials will allow more accurate cyst detection. Water samples have been filtered using polypropylene filters (Jakubowski, 1984) and polycarbonate filters (Ongerth, 1987), with both filter types demonstrating significant cyst recovery. Once cysts have been concentrated from water samples, they need to be separated from other particles in the sample. This has been attempted using zinc-sulfate (LeChevallier et al., 1990), potassium citrate (Ongerth and Stibbs, 1987), Percoll-Sucrose gradients (Jakubowski, 1984), and propidium iodide (Sauch et al., 1991). The zinc-sulfate and propidium iodide

methods were shown to be time-consuming and fatiguing, not only because of the low numbers of *Giardia* cysts found among the abundance of other microorganisms and debris, but also because of the poor visual contrast between *Giardia* cysts and other contaminants in the sample. *Giardia* has been detected most readily using immunofluorescent antibody (IFA) and Percoll-Sucrose gradients (Sauch, 1984; Sauch, 1985). The cysts fluoresced bright green and could be quickly located even in samples heavily contaminated with other microorganisms and debris. Ease of isolation and detection of cysts will allow for more accurate detections of *Giardia* cysts in both raw and treated water supplies.

Methods for filtration, elution, clarification, and detection of *Cryptosporidium* in water are similar to those for *Giardia* (Rose et al., 1989). The present study used the IFA procedure for detection of *Giardia* cysts and *Cryptosporidium* oocysts.

### **Laboratory Accuracy for *Giardia* and *Cryptosporidium***

#### **Detection**

While advancements are continually being made for the accurate detection of *Giardia* and *Cryptosporidium*, a recent survey questions how accurately laboratories are utilizing current techniques. A blind survey of 16 commercial labs was conducted to test for accuracy in *Giardia* and *Cryptosporidium* analysis using the American Society for Testing and Material (ASTM) methods (Clancy et al., 1994). Spiked samples were sent to laboratories for analysis. *Giardia* cyst recoveries

ranged from 0.8 to 22.3%, averaging only 9.1%; *Cryptosporidium* oocysts recoveries ranged from 1.3 to 5.5%, averaging 2.8%. Four of the laboratories falsely reported the *Oocystis* algae as *Giardia*, four laboratories failed to recover *Giardia* cysts, and six laboratories failed to recover the *Cryptosporidium* oocysts. Not all laboratories strictly followed the ASTM method. Clancy et al. (1994) concluded that a majority of labs need to make improvements in one or more of the following areas: response to clients; adequacy of sampling equipment and directions for use; analytical methods; and data accuracy. Additional research and testing need to be conducted for laboratories to become more proficient in isolating and identifying *Giardia* and *Cryptosporidium* from raw and drinking water sources.



## Chapter III

### Materials and Methods

Water samples used in this study were collected between May and September, 1994 from four select Water Treatment Plants:

1. Plant #1 (Figure 8 and Table 3)
2. Plant #2 (Figure 9 and Table 4)
3. Plant #3 (Figure 10 and Table 5)
4. Plant #4 (Figure 11 and Table 6)

The procedures utilized in this research are those published in the Federal Register-EPA 59, 1994.

#### **Sample Apparatus Preparation and Assembly**

A filter housing (Filterite, Timonium, MD, Model 910088-000) was used to collect water samples. It consisted of an inlet hose, filter holder, a one  $\mu\text{m}$  nominal porosity polypropylene filter, an outlet hose, a water meter, and a flow control valve (Figure 12). A pump was used on samples with an unpressurized water source. The sampling apparatus was not sterile but was clean and free of cysts and oocysts. In order to insure a clean, uncontaminated sampling apparatus, all parts of the apparatus were thoroughly rinsed with at least 50 gallons (190 liters) of the water to be sampled prior to the installation of the filter cartridge. If multiple samples had to be collected at the same time with the same apparatus, the sample collections were collected beginning with the least

Table 3 Plant #1 Servicing Information

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Source Water:	River
# People Served:	15,000
Water Processed / 24 Hours:	2.50 - 3.00 million gallons
Average NTU:	0.08 - 0.15
Chlorine Dosage:	3.80 ppm (parts per million)
Chlorine Contact Time:	3.5 hours

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Table 4 Plant #2 Servicing Information

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Source Water:	Creek
# People Served:	10,000
Water Processed / 24 Hours:	400,000 - 500,000 gallons
Average NTU:	0.30 - 0.40
Chlorine Dosage:	0.70 - 0.80 ppm
Chlorine Contact Time:	4.5 hours

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Figure 8 Plant #1 Schematic Flow Diagram

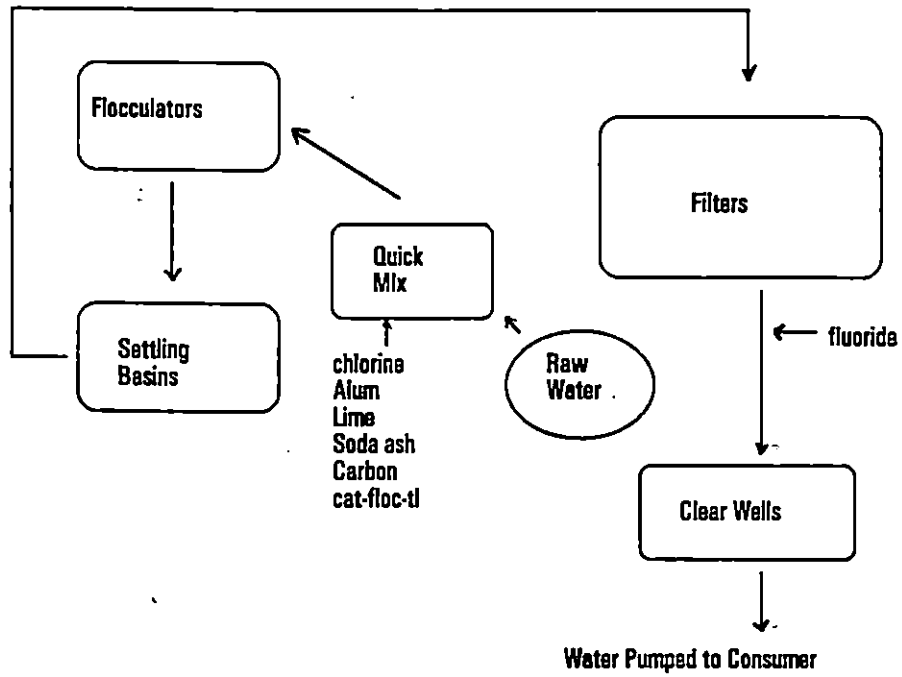


Figure 9 Plant #2 Schematic Flow Diagram

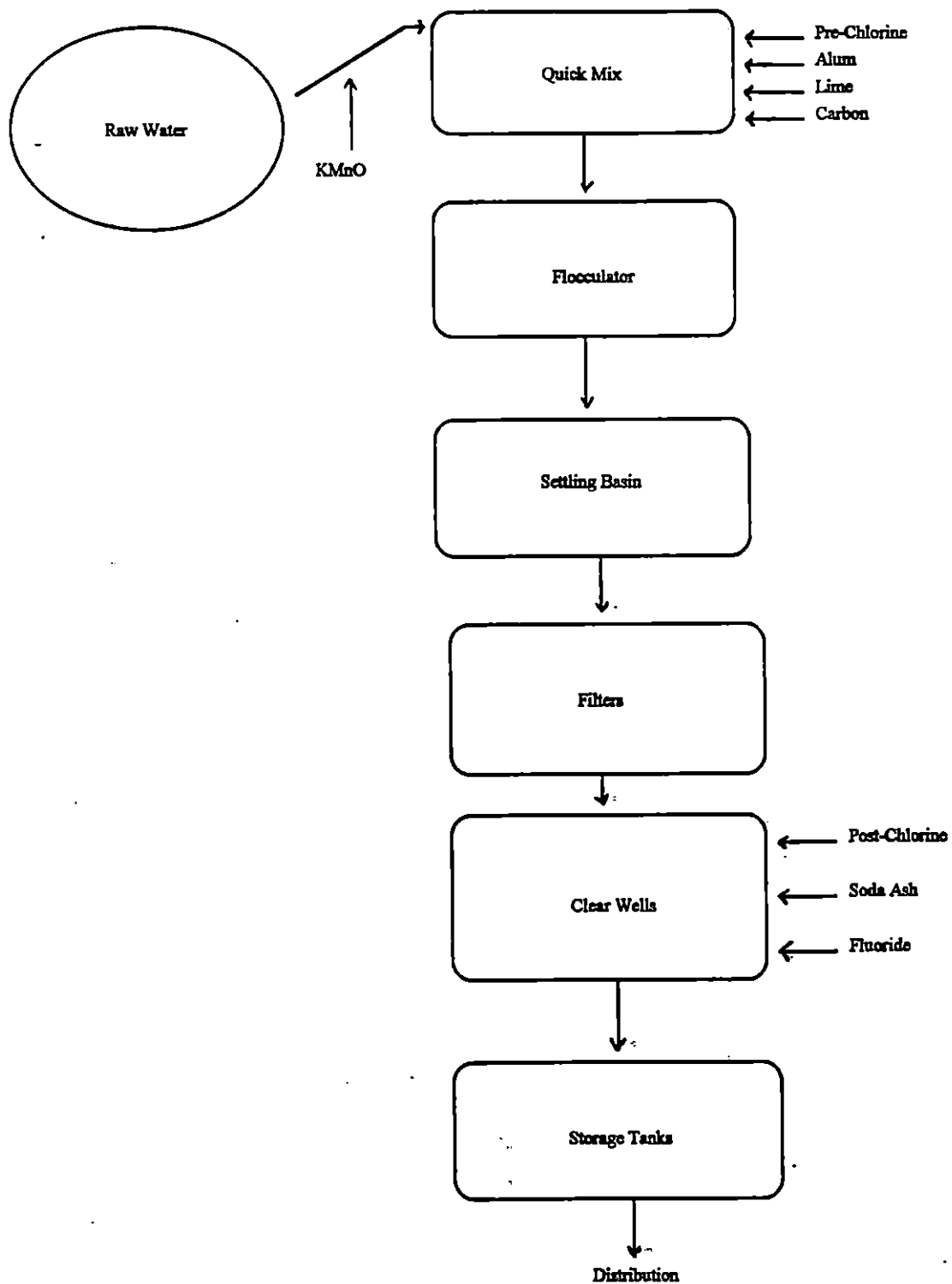


Table 5 Plant #3 Servicing Information

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Source Water:	Reservoir
# People Served:	5,400
Water Processed / 24 Hours:	600,000 gallons
Average NTU:	0.20 - 0.30
Chlorine Dosage:	1.50 - 2.00 ppm
Chlorine Contact Time:	4.5 hours

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Table 6 Plant #4 Servicing Information

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Source Water:	Creek
# People Served:	13,500
Water Processed / 24 Hours:	1,800,000 gallons
Average NTU:	0.10
Chlorine Dosage:	2.00 ppm
Chlorine Contact Time:	4.78 hours

---

Figure 10 Plant #3 Schematic Flow Diagram

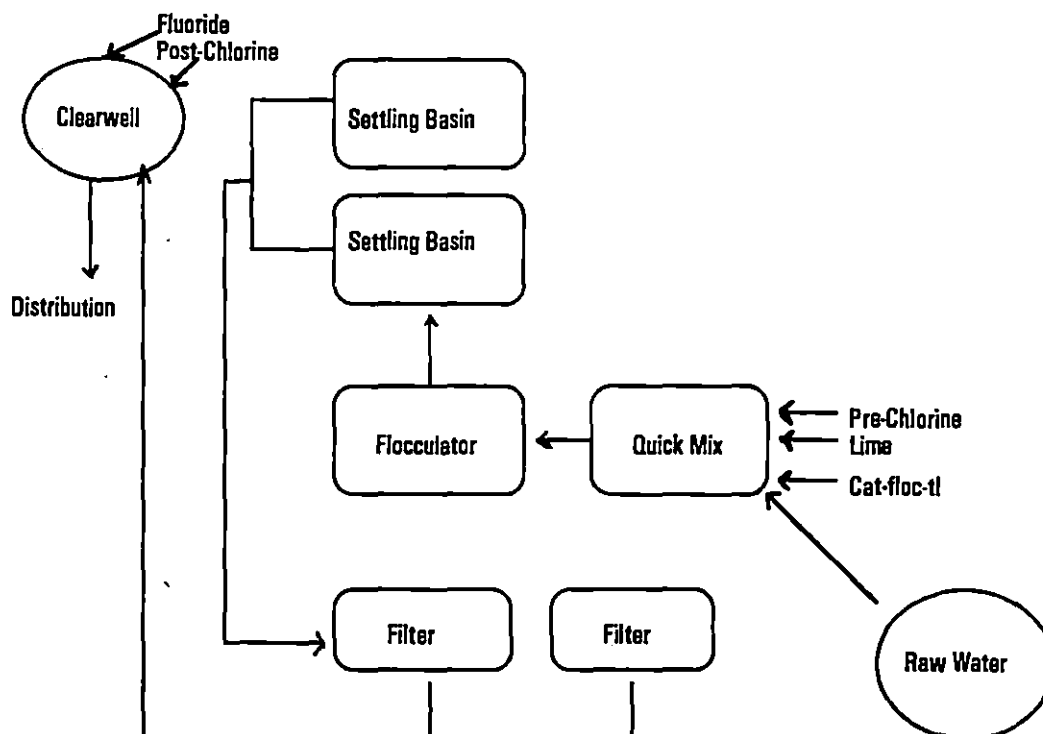


Figure 11 Plant # 4 Schematic Flow Diagram

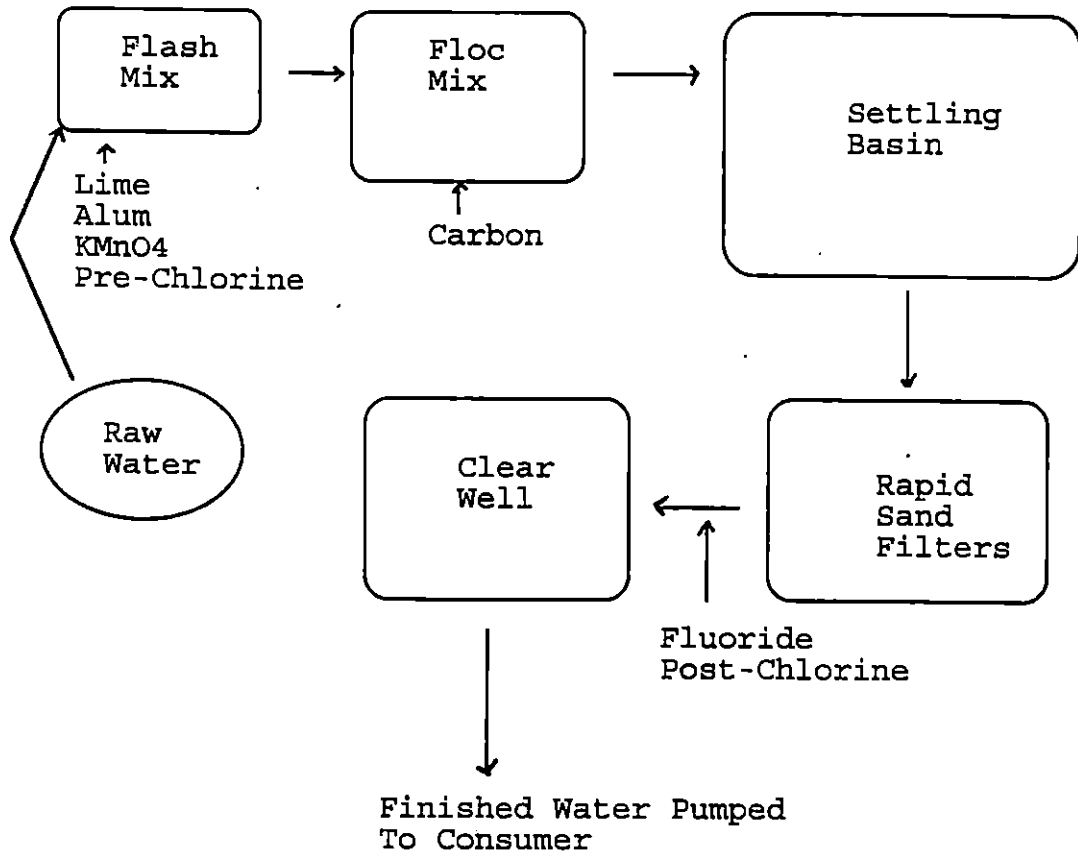
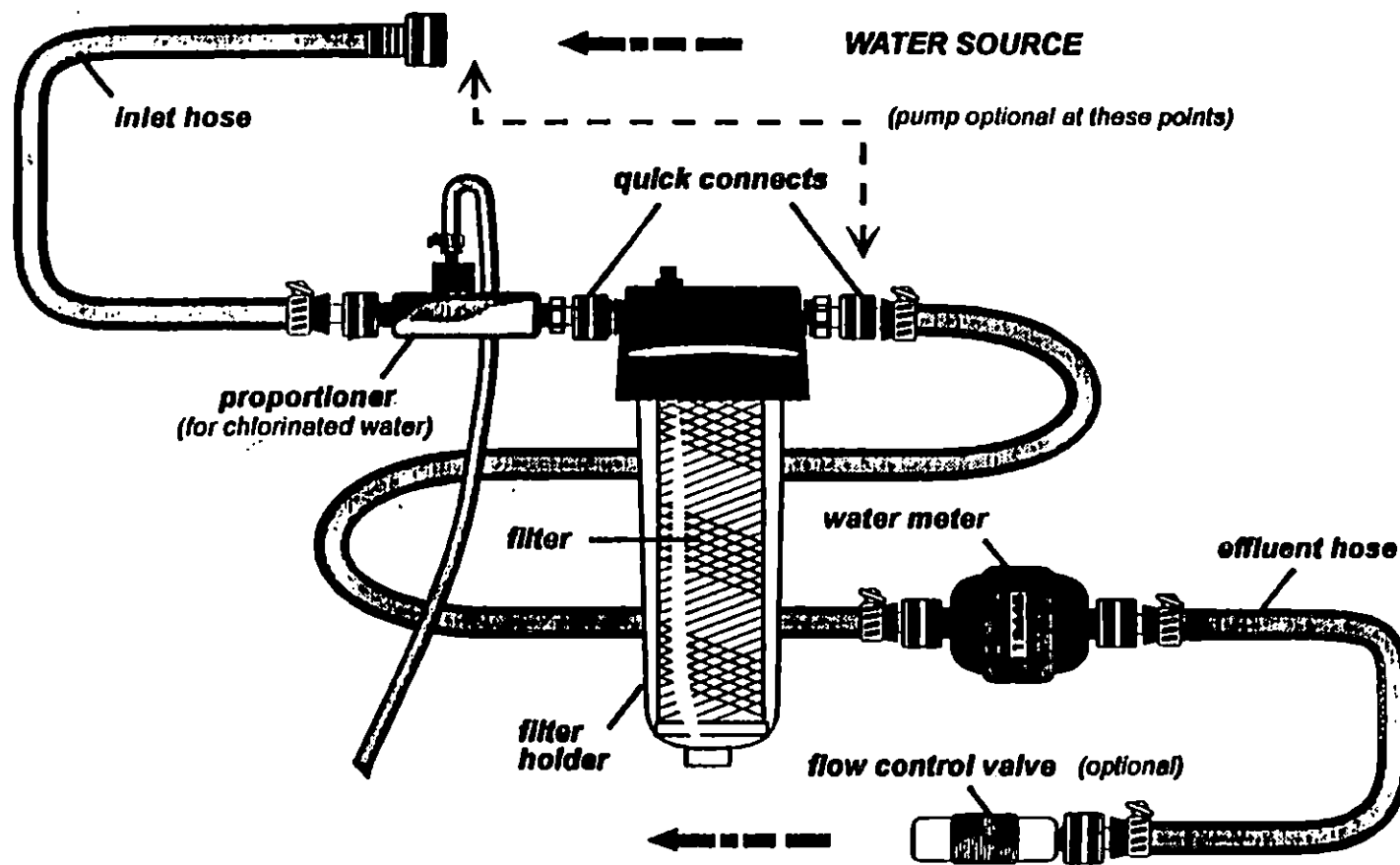


Figure 12 Filter Housing Set-Up — Collection Apparatus



\*EPA Federal Register



contaminated water (e.g. treated water) and ending with the most contaminated water (e.g. source water).

Inlet and outlet hoses for the filter holder consisted of standard garden hoses and fittings. Outlet hoses were used repeatedly without washing between samples but the inlet hoses were contaminated after one use and were rinsed thoroughly with at least 50 gallons of the water to be sampled prior to connecting the filter holder. If a pump was used to collect the sample, it was placed on the outlet end of the sampling apparatus in order to avoid washing between sample collections.

### **Sample Collection**

Raw and treated water samples were collected at the same time from each treatment facility. A minimum sample of 100 gallons for raw water and 1,000 gallons for treated water were filtered through a ten inch (25.5 cm) wound polypropylene cartridge filter having a nominal porosity of one  $\mu\text{m}$  (Filterite Corp., Timonium, MD). Separate filtering systems were used for raw and filtered samples. If the filtered water had been treated with chlorine or other disinfectants, the sample was neutralized with a 0.5% sodium thiosulfate solution [0.5g sodium thiosulfate (Fisher Scientific, Fair Lawn, NJ) and 100 mL distilled, deionized water]. One liter of 0.5% sodium thiosulfate solution was needed for each 100 L of sample collected.

After collection, the filters (along with their residual water remaining in the filter holders) were placed in Whirl-Pak bags (Nasco, CMS Scientific). The bags were sealed and placed in another Whirl-Pak

bag. The double bagged samples were immediately transported to the laboratory on ice packs in an ice chest then stored at 2-5 °C to be processed within 24-72 hours.

Using sterile bottles, approximately 200 mL of both raw and treated water were collected to be used later for fecal coliform analysis. Turbidity and pH readings were provided by water plant personnel for both raw and finished drinking water supplies.

### **Sample Processing**

To prepare the samples for analysis, the residual water from each filter was poured off and saved for later use. The filters were cut lengthwise to the core, using a sterile surgical scalpel to produce fibers approximately 2 inches long. After teasing the fibers apart, each filter was placed in a 3,500 mL capacity sterile Stomacher bag (Tekmar Co., Cincinnati, Ohio) along with 1.75 L of an eluting solution. The filter material was homogenized for two five-minute intervals in a Stomacher Lab Blender, Model 3500 (Tekmar Co., Cincinnati, OH). Between the homogenization periods, the filters were hand kneaded to redistribute the fibers in the bag. After homogenization, the filter material was hand wrung using sterile gloves to remove as much of the eluant liquid as possible. Filter material was visually inspected before being discarded to ensure that no debris remained on the filter. Fibers containing debris were rewashed.

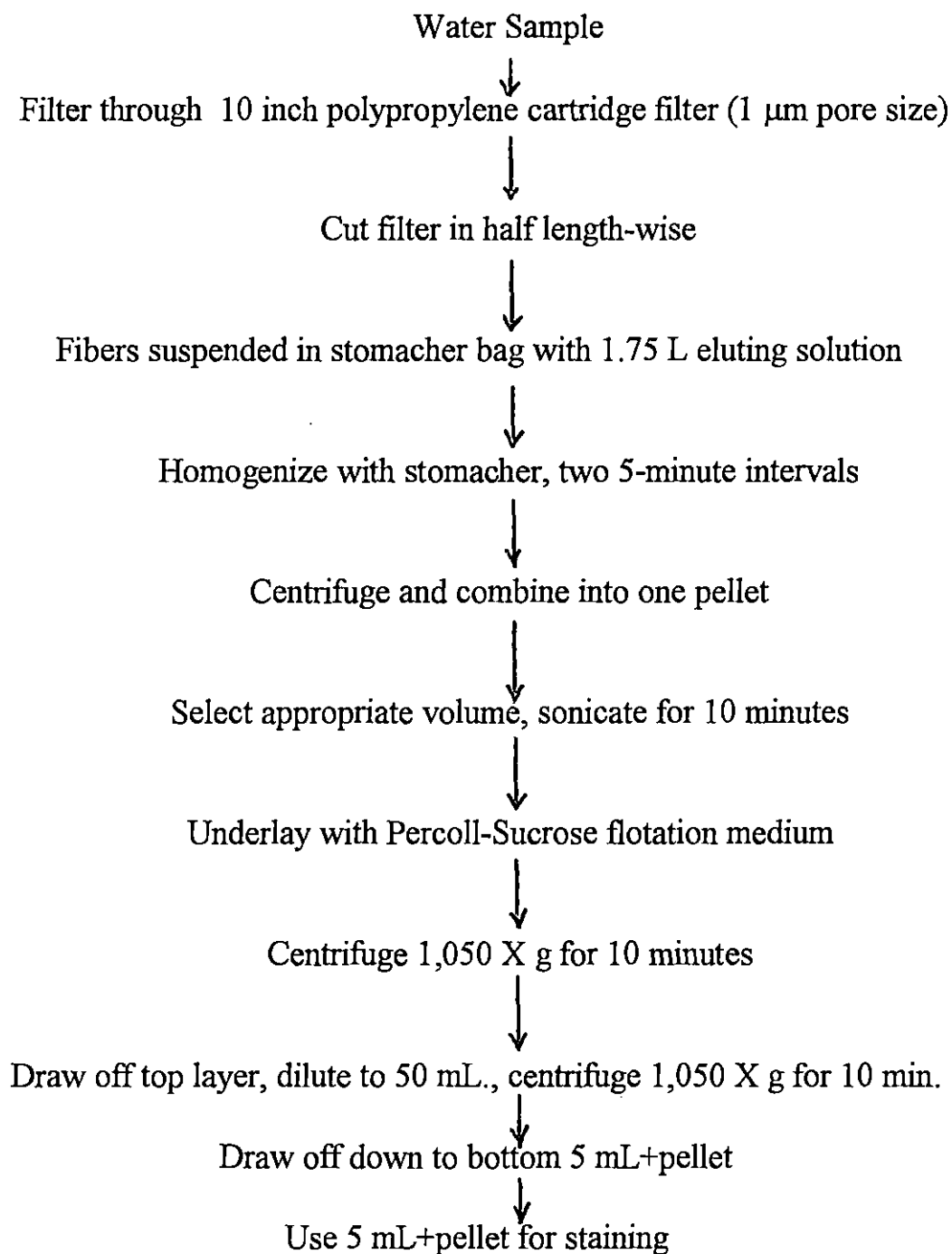
The eluant liquid from each filter was combined with its residual water. The mixture was then poured into 250 mL centrifuge bottles

(Fisher), and concentrated into single pellets by centrifugation at 1,050 x g for 10 minutes using a swinging bucket rotor, in a refrigerated, superspeed centrifuge, Model 2K (International Equipment Company, Needham HTS, Mass). The supernatant fluid was aspirated and the pellets from each bottle were resuspended by vortexing using a Maxi-Mix Mixer, Type 16700 (Fisher). The pellets were then combined into one bottle and the volume of the packed pellet was recorded. The pellet was suspended in an equal amount of 10% neutral buffered formalin solution. If the final pellet volume was less than 0.50 mL, enough buffered formalin was added to bring the resuspended pellet volume to 1.0 mL.

All raw water samples ran were archived. A maximum of 5 mL of the packed pellet and formalin suspension was transferred into a 50 mL tube with the following information affixed: the water plant facility, date of collection, and type of sample. All archived samples were stored at 4 °C.

In a clear plastic 50 mL conical centrifuge tube, a 1.0 mL sample of the resuspended pellet was combined with 19 mL of eluting solution and vortexed for 10 minutes. Using a 50 mL syringe and 14 gauge cannula, the vortexed suspension was underlayered with 30 mL of a Percoll-Sucrose Flotation Medium. The specific gravity of the Percoll-Sucrose Medium was measured with a Hydrometer (Fisher Scientific, Fair Lawns, NJ) and was used only if the specific gravity was between 1.09 and 1.10. The suspension was then centrifuged at 1,050 x g for

FIGURE 13 Method For Preparation of *Giardia* Cysts and *Cryptosporidium* Oocysts From Water



\*LeChevallier et al., 1991

10 minutes. Using a polystyrene 25 mL pipet rinsed with the eluting solution, the top layer (containing cysts or oocysts), the interface, and 5 mL of the Percoll-Sucrose Medium below the interface was drawn off and placed in a 50 mL conical centrifuge tube. The liquid was then diluted to 50 mL with the eluting solution and recentrifuged at 1,050 x g for 10 minutes. The supernatant fluid was drawn off down to 5 mL (plus pellet) and vortexed. This suspension was saved for future processing with fluorescent antibody reagents (Figure 13).

### **Labeling Procedure**

Sample concentrates were added to filters using a Hoefer Manifold (Hoefer Scientific, San Francisco, CA). Twenty-five mm cellulose acetate filters (0.20  $\mu\text{m}$  pore size; Sartorius Inc., Haywood, CA) and 25 mm Durapore HVLP filters (0.45  $\mu\text{m}$  pore size; Millipore Corp., Bedford, Maine) were presoaked in PBS (phosphate buffered saline, 1X strength) for at least one minute before being placed on the manifolds of the Hoefer. The Durapore HVLP filters were placed under the cellulose acetate filters to ensure even distribution of sample. With both filters on the Hoefer, a vacuum pump (Model XX5500000, Millipore) was then turned on and the manifold well support valves were open to flatten the filters to the manifolds. A rubber policeman was used to remove any air bubbles trapped under the filters and to remove any creases or wrinkles on any of the membrane filters. With the stainless steel wells firmly placed over each filter and the manifold support valves open, the inside of each well was rinsed with 2 mL 1% bovine serum

albumin (BSA) with a Pasteur pipet. The BSA was completely drained from the wells and the manifold valves were closed. Sample volumes, typically 0.5 to 1.5 mL for raw samples and 1.0 to 2.0 mL for treated samples, were used to provide a monolayer of material on the filter surfaces.

### **Antibody Quality Control**

Positive and negative antibody controls were run every time samples were filtered through the Hoefer. Negative controls consisted of 1.0 mL of PBS while positive controls consisted of 100  $\mu$ L of a positive control from a Meridian Hydrofluor-Combo Kit for *Giardia* and *Cryptosporidium* (Cat# 240025, Lot K0210017, Meridian Diagnostics, Cincinnati, Ohio). Performance evaluation samples were also processed as positive controls (Parasitology Research Lab, Phoenix, Arizona). The manifold valves under each membrane filter were opened to drain the wells. Each well was then rinsed with 2 mL 1% BSA making sure not to touch the sides of the wells. The BSA helped to eliminate nonspecific binding of the antibodies to microorganisms or debris other than *Giardia* or *Cryptosporidium*. The manifold valves under each membrane filter were then closed.

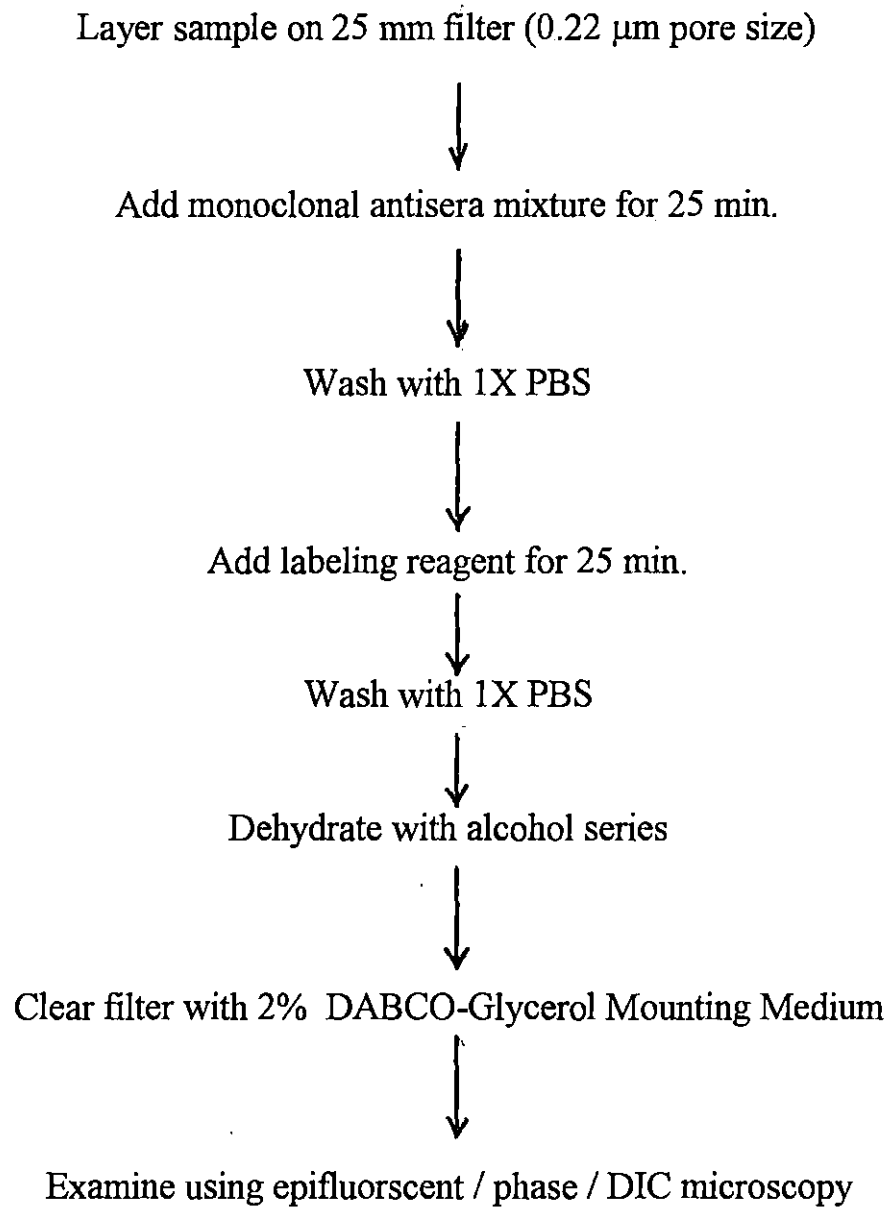
### **Indirect Fluorescent Antibody Staining**

Monoclonal antisera that was specific for *Giardia* and *Cryptosporidium* (Hydrofluor-Combo Kit, Meridian Diagnostics Inc.) was diluted according to the manufacturer's instructions using PBS. A

primary antibody (0.5 mL; lot# CG21S017) was pipetted onto each membrane filter and allowed to remain in contact for 25 minutes at room temperature. The primary antibody was specific for surface antigens on the cell wall of *Giardia* and *Cryptosporidium* and would bind only to those cells. At the end of the contact period, the antisera was drained from the wells and each well and filter were rinsed 5 times with 2 mL of PBS. A labeling reagent (0.5 mL; lot# MM21S017) was then added to the filters and allowed to react for 25 minutes. The labeling reagent bound specifically to the primary antibody that has bound to the surface of the *Giardia* cysts and *Cryptosporidium* oocysts. The labeling reagent will cause the cells to demonstrate an apple-green fluorescence. During this contact period, all wells were covered with aluminum foil to shield the reagents from light and to prevent dehydration and crystallization of the fluorescein isothiocyanate dye. At the end of the contact period, the labeling reagent was drained and the filters were rinsed with 2 mL PBS.

The membrane filters were dehydrated by applying 1 mL of 10, 20, 40, and 80 % ethanol /glycerol alcohol solutions. An eriochrome Black T counterstain (Analytical Services, Essex Junction, VT) was then added to each filter. One milliliter was added to the filters and allowed a contact time of 30-60 seconds. All solutions were drained from the filters. Using different forceps with each filter, the 0.20 pore size filters were then placed on prewarmed, labeled microscope slides containing 75  $\mu$ L DABCO-Glycerol mounting medium. If, upon adding the filters to the slides, portions of the filters became white and not saturated with

FIGURE 14 INDIRECT FLUORESCENT ANTIBODY STAINING  
OF *GIARDIA* AND *CRYPTOSPORIDIUM*



\*LeChevallier et al., 1991



the mounting medium, the filters were lifted and additional DABCO mounting medium was added to the slide. The filters were allowed to clear for 20 minutes on the microscope slides. At the end of the 20 minute clearing period, the filters appeared drier and became transparent. Twenty  $\mu\text{L}$  of the DABCO mounting medium was then applied on top of the filters and a 25 mm X 25 mm coverslip was placed over the filters. The coverslip was tapped with the handle of a pair of forceps to remove any trapped air bubbles. The excess DABCO-Glycerol mounting medium was wiped away from the edge of the coverslip with a moistened Kimwipe. The edges of the coverslip were sealed using clear fingernail polish and the slides were stored in a box filled with Drierite at 4 °C. Slides were observed within five days of their preparation (Figure 14).

### **Microscopic Examination**

Microscopic examination of *Giardia* cysts and *Cryptosporidium* oocysts was performed on a Zeiss Axioscope, Model G 42-110-e (Carl Zeiss, D-7082 Oberkochen, West Germany). The research grade microscope was equipped with bright and darkfield illumination, phase-contrast microscopy, differential interface contrast (DIC) microscopy, and fluorescence microscopy. Slides were scanned on the 40X objective while using fluorescent microscopy. All measurements were performed on the 100X objective utilizing a VIA-100 Video Monitor Measurement System (Zeiss) and Sony Color Video Monitor, Model

Figure 15 Diagram of Giardia spp. Trophozoite

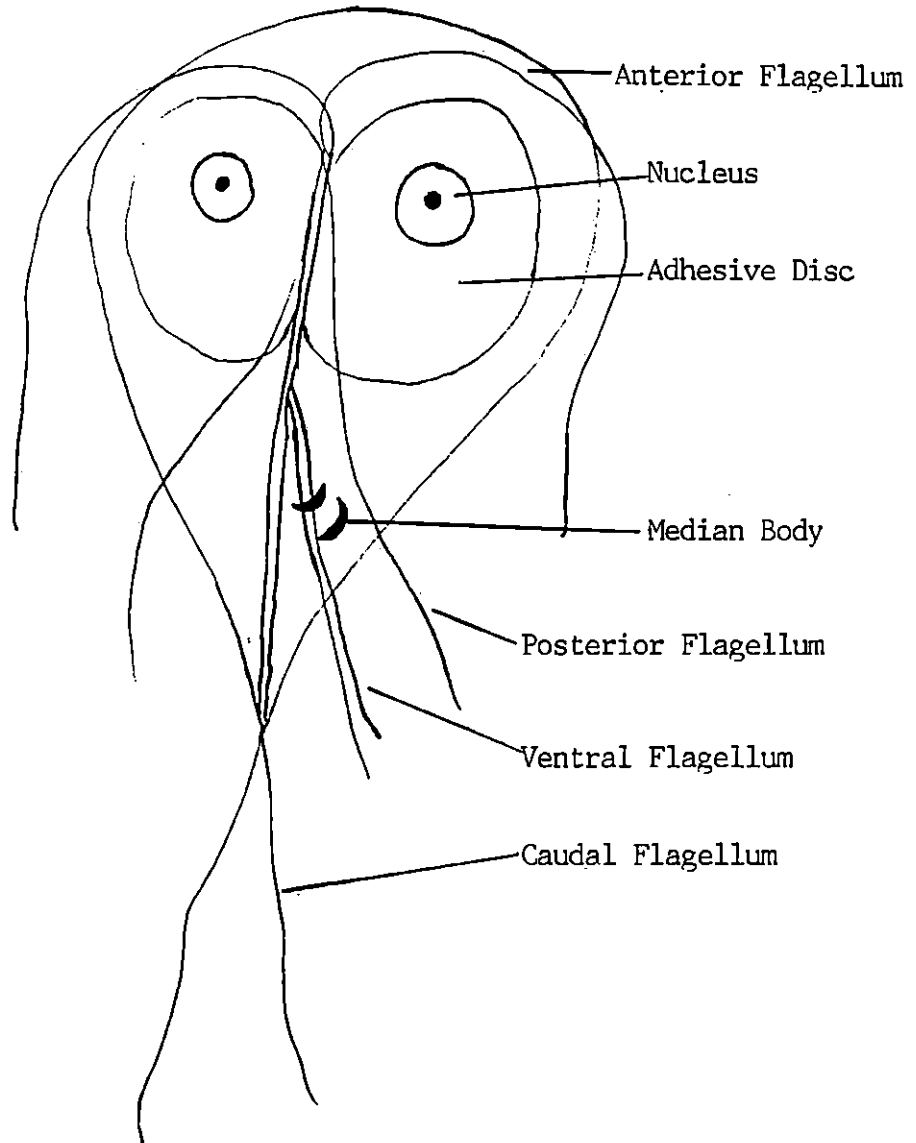


Figure 16 Diagram of Giardia spp. Cyst

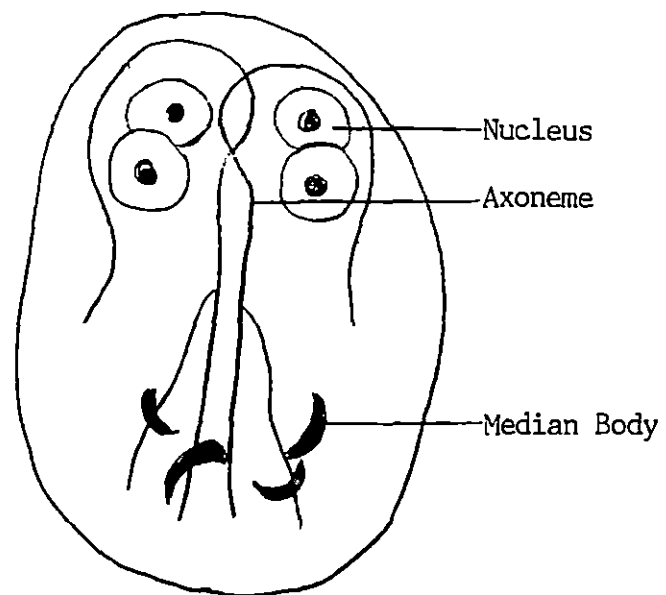
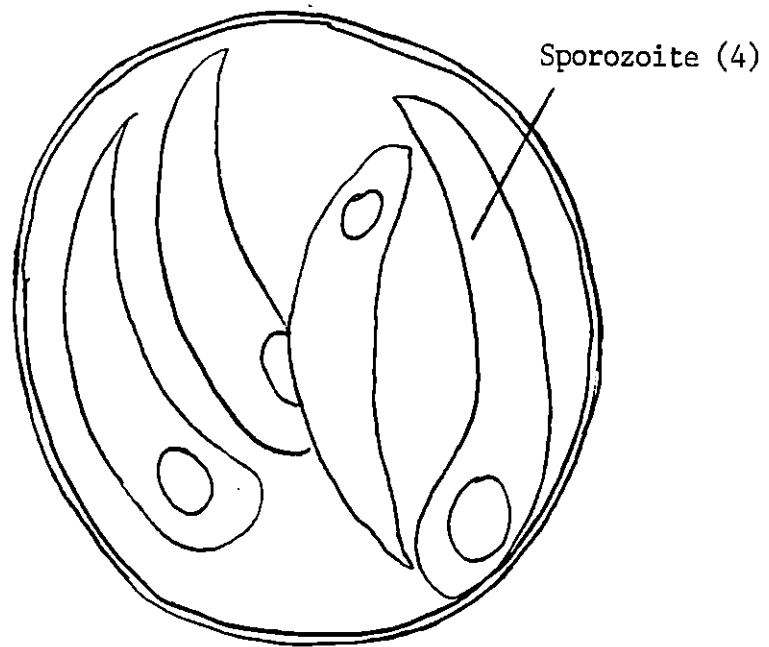


Figure 17 Cryptosporidium parvum Oocyst



PVM-1343MD (Zeiss). All photography was performed using a CCD Video Camera System, Optronics Model ZVS-47EC (Zeiss).

*Giardia* cysts were presumptively identified by the cyst size ( 8-14  $\mu\text{m}$  in length and 7-10  $\mu\text{m}$  in diameter) (Lin, 1985), fluorescence, and shape. *Giardia* cysts were confirmed by phase-contrast and DIC microscopic observations of internal structures at 1,500X magnification.

*Giardia lamblia* cysts exhibit three prominent internal structures: 1) axoneme - an internal flagellar structure, 2) median bodies - prominent, dark-staining, paired organelles in the posterior of the cyst often having a claw-hammer shape, and 3) nuclei - prominent internal structures often ranging from 2-4 per cysts (Figure 16). *Giardia* cysts confirmation required the presence of at least two of these internal structures. Cyst viability was determined using DIC microscopy.

Presumptive *Cryptosporidium* oocysts were determined by size (4-6  $\mu\text{m}$ ) (Carcia and Bruckner 50), shape, and surface texture. Confirmed oocysts exhibited surface folds or one to four infectious, motile, banana-shaped sporozoites within the oocysts as observed by phase-contrast or DIC microscopy (Figure 17).

Cyst/oocyst densities were calculated as number/100 L for both surface water and tap water (Figure 18) and recorded per L. Upon zero parasite density detection, the parasite level will be reported as less than the detection limit. When no cysts or oocysts were found, an estimated value was determined and indicated by the use of (<) before the levels.

Figure 18 Calculation Formula for Determining *Giardia* and *Cryptosporidium* / 100 L Water

$$\frac{X}{100} = \frac{(\text{PRG or PRC or CG or CC}) \times 100}{\text{FVR}}$$

PRG or PRC = Presumptive *Giardia* or *Cryptosporidium*

CG or CC = Confirmed *Giardia* or *Cryptosporidium*

F = Fraction of packed pellet volume (P) subjected to flotation

$$\begin{array}{r} \text{Ex. 1 mL} \quad \text{Floated} \\ \hline 30 \text{ mL} \quad \text{Total packed pellet volume} \end{array}$$

V = Volume (Liters) of original water sample  
(Sample amount ran through filter)

R = Percent (Expressed as a decimal) of floated sediment  
examined

Ex. 25 mL floated and 1.0 mL examined

$$\frac{1.0 \text{ mL}}{25 \text{ mL}} = 0.04 \text{ or } 4\% \text{ examined}$$

Example Problem: 4 confirmed organisms  
1.0 mL floated from 25 mL pellet  
100 Liters processed  
0.5 mL examined microscopically

$$\frac{X}{100 \text{ L}} = \frac{4 (100)}{1.0 / 25 \times 100 \text{ L} \times 0.5 / 25} = 5,000 / 100 \text{ L}$$

### **m-Fc Media Preparation**

m-Fc media was used to determine fecal coliforms in both treated and raw water samples. Using strict aseptic technique, 3.7 grams of m-Fc dehydrated media (Difco), 1.0 gram Rosolic acid (Matheson Coleman and Bell), and 100 mL distilled water were combined and stirred into solution over low heat using a hot plate (Corning Stirrer/Hotplate). The media was then sterilized in an Amsco Eagle 2011 Gravity Autoclave (American Sterilizer Company, Pittsburg, PA) at 15 psi for 15 minutes. After the media was sterilized, it was cooled to 50 °C in a hot water bath. The m-Fc broth media was stored at 4 °C. Also, prepared ampules of m-Fc media (HACH, Loveland, Colorado, cat. no. 23732-20) were utilized.

### **Fecal Coliform Procedure**

Fecal coliform analysis was performed on both raw and treated water samples. Plastic Millipore plastic plates (Ct. No. pd104700) were opened and filter pads were placed inside. Using a sterile pipet, 2 mL of the m-Fc media or one ampule of the prepared HACH m-Fc media was placed on each plate.

Six millipore funnels were placed in a UV sterilization box (Millipore, Cat. No. XX6370000). Approximately 2-3 minutes was required for sterilization of the funnels. Using flamed forceps, membrane filters (Millipore, 45 µm, HAWG04752) were placed in each unit of a 3-place manifold and 3 sterile funnels were placed upon the manifolds. Funnels were screwed on to avoid spills of the water

samples. Each sample was shaken for approximately 25 seconds. The samples (10 mL for raw; 100 mL for finished water) were poured into the assembly, rinsing the funnels twice with 30 mL of sterile water.

Using sterile forceps, the membrane filters were removed and placed on a pad saturated with the m-Fc broth media. The samples were incubated at 44.5 °C for 22-24 hours and fecal coliform colonies exhibited a blue color. Fecal coliforms were computed per 100 mL.



## Chapter IV

### Results

Eight samples of both raw and finished water from selected water treatment facilities in Eastern Kentucky were collected in an attempt to demonstrate the presence of two pathogenic protozoa, *Giardia lamblia* and *Cryptosporidium parvum* (Table 7; Figures 19-21). Of the eight raw water samples, 88% (seven of eight) were positive for both protozoa. Distribution ranges represent observed protozoan levels at each sample site and source. *Giardia lamblia* cysts were isolated in 50% (four of eight) of the samples with a distribution range of <0.629-1.468 cysts/L water. *Cryptosporidium parvum* oocysts were more widespread being present in 63% (five of eight) raw samples ranging between 0.315 - 2.642 oocysts/L. Both *Giardia* and *Cryptosporidium* were identified in only 25% (two of eight) of the raw water samples. Of the eight finished water samples collected, 25% (two of eight) were positive for pathogenic protozoa. *Giardia* (estimated <0.0013 - <0.106 cysts/L) were not isolated from any treated sample. *Cryptosporidium* oocysts were isolated in 25% (two of eight) treated water samples (<0.0013 - 0.1206 oocysts/L).

#### **Plant #1 Detection Results**

Four samples were collected at each of four plants (two raw; two finished). In Plant #1, 50% (one of two) of the raw water samples were positive for *Cryptosporidium* (0.315 - 0.661 oocysts/L). *Giardia* cysts

were found in 50% (one of two) of the raw samples (<0.629 - 1.32 cysts/L).

Two finished water samples were collected from Plant #1. Neither *Giardia* nor *Cryptosporidium* were detected in treated water. However, a distribution range of <0.035 to <0.053 organisms/L water was predicted for both protozoans using the formula in Figure 18.

On May 16, 1994, Plant #1 demonstrated a removal rate of 92% for *Giardia* cysts and 83% for *Cryptosporidium* oocysts. On July 6, 1994, removal rates increased slightly showing 97% removal of *Giardia* cysts and 95% removal of *Cryptosporidium* oocysts.

### **Plant #2 Detection Results**

*Giardia* cysts were isolated in 50% (one of two) raw water samples in Plant #2 (<0.881-1.468 cysts/L). *Cryptosporidium* oocysts were more numerous in both raw samples (0.734-2.642 oocysts/L).

One of the two finished water samples (50%) collected at Plant #2 demonstrated the presence of *Cryptosporidium* oocysts (<0.054-0.062 oocysts/L). The raw water sample ran on June 6, 1994 revealed *Cryptosporidium* oocysts levels of 0.062 oocysts/L finished water. Based on oocysts levels in raw water samples (0.734 oocysts/L) processed the same day, a 92% removal rate was achieved by the plant's filtration practices. This removal rate falls below the 99.9% removal rate that was specified in the Surface Water Treatment Rule (See Introduction). *Giardia* cysts were not shown to be present in the finished water but were predicted to have a distribution rate of <0.031 to

<0.054 cysts/L water. While no organisms were detected in the finished water on July 12, 1994, only a 96% and 99% removal rate was shown for *Giardia* and *Cryptosporidium*, respectively.

### **Plant #3 Detection Results**

In Plant #3, *Giardia* cysts were found in one of two raw water samples (0.44-1.536 cysts/L). *Cryptosporidium* oocysts were not found in either raw sample (<0.44-<1.536 oocysts/L). Water processed by Plant #3 was free of both *Giardia* and *Cryptosporidium* (<0.0013-<0.106 cysts and oocysts/L). On June 14, 1994, *Giardia* and *Cryptosporidium* removal rates were shown to be 99%. Removal rates dropped on July 18, 1994, showing only a 93% removal for both *Giardia* and *Cryptosporidium*.

### **Plant #4 Detection Results**

The final 4 samples were collected from the water Plant #4. Raw water demonstrated a large distribution of pathogenic protozoa. *Giardia* cysts were found in 50% (one of two) of the raw water samples (0.901-1.10 cysts/L) while *Cryptosporidium* oocysts were more prevalent in 100% (1.811-2.202 oocysts/L) of the raw water samples. Oocysts were demonstrated in 50% (one of two) finished samples (<0.0348-0.1206 oocysts/L). On September 26, 1994, the *Cryptosporidium* oocyst level in treated water was shown to be 0.1206 oocysts/L. Based on a oocyst level of 1.811 oocysts/L in a raw water

Table 7 Summary of *Giardia* and *Cryptosporidium* / L Detected in Raw and Finished Water Sources From Select Treatment Plants in Eastern Kentucky

Date	Plant	Raw Water		Finished Water		% *
		<i>Giardia</i>	<i>Cryptosporidium</i>	<i>Giardia</i>	<i>Cryptosporidium</i>	
5-16-94	#1	<.629	.315	<.053	<.053	92&83
7-6-94	#1	1.32	<.661	<.035	<.035	97&95
6-6-94	#2	1.468	.734	<.054	.062	96&92
7-12-94	#2	<.881	2.642	<.031	<.031	96&99
6-14-94	#3	.440	<.440	<.0013	<.0013	99&99
7-18-94	#3	<1.536	<1.536	<.106	<.106	93&93
9-20-94	#4	<1.10	2.202	<.0348	<.0348	97&98
9-26-94	#4	.901	1.811	<.0905	.1206	80&93

\*\*Indicated removal rates based on estimated values of *Giardia* and *Cryptosporidium* respectively. Cyst removal rates are shown first followed by oocyst removal rates.

Figure 19 *Giardia* Cysts Distribution Between Select Raw Water Sources in Eastern Kentucky

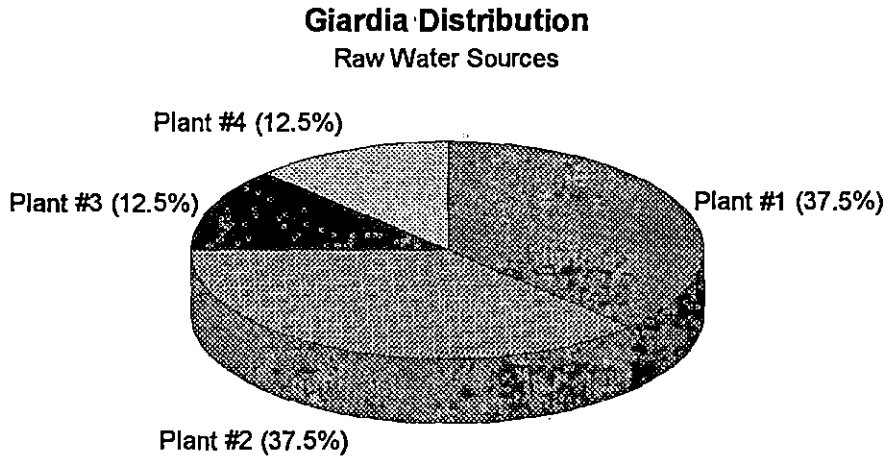


Figure 20 *Cryptosporidium* Oocyst Distribution Between Select Raw Water Sources in Eastern Kentucky

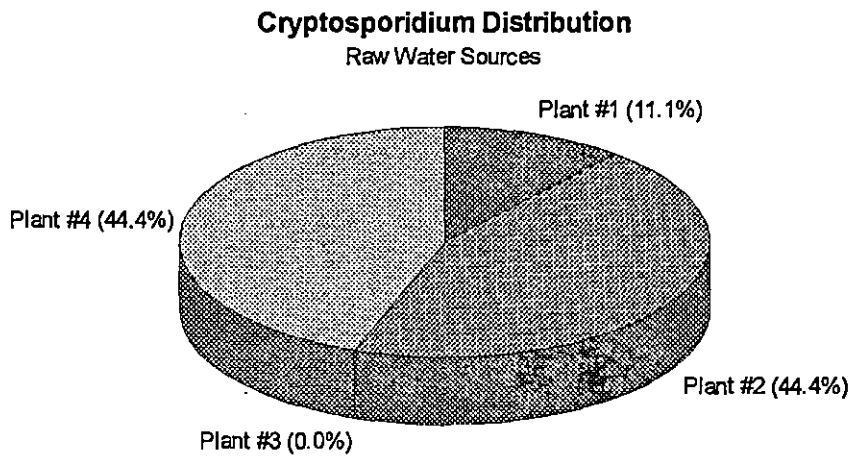
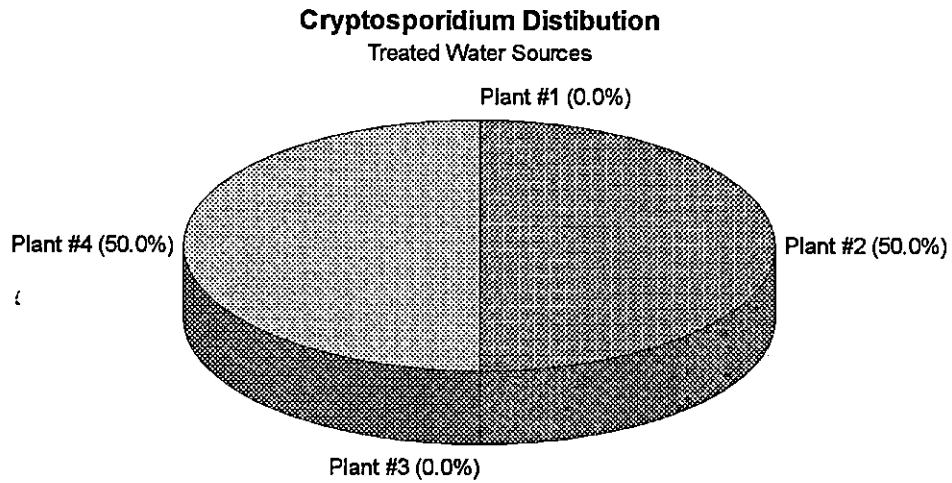


Figure 21 *Cryptosporidium* Oocyst Distribution Between Select Treated Water Sources in Eastern Kentucky



sample processed the same day, the plant was achieving a 93% oocyst removal rate. *Giardia* cysts, range <0.0348 to <.0905 cysts/L, were not observed in treated samples. Removal rates on September 20, 1994, were 97% for *Giardia* cysts and 98% for *Cryptosporidium* oocysts.

### **Correlations of Protozoa in Finished and Raw Water**

Past studies have shown that concentrations of *Giardia* and *Cryptosporidium* in raw water may influence the protozoan levels seen in treated waters (LeChevallier and Norton, 1991). Using the SAS statistics package (SAS Institute), correlation between *Giardia lamblia* in raw and treated water samples showed a  $r = 0.53$  correlation between cysts numbers. At a 95% confidence level, this correlation proved to be insignificant ( $p=0.17$ ).

Similar results were seen on a correlation analysis between raw and finished water levels of *Cryptosporidium parvum*. A poor relationship was observed between raw and treated oocysts levels ( $r = 0.32$ ;  $p = 0.48$ ).

### **Effects of pH on *Giardia* and *Cryptosporidium* Levels**

pH readings were obtained from water plant officials on all raw water samples in an effort to determine if pH correlated with numbers of *Giardia* cysts and *Cryptosporidium* oocysts found in raw and finished water sources (Figures 22-23; Tables 8-9). *Giardia* cysts and pH in raw water samples had a slight but insignificant correlation ( $r=0.66$ ;  $p=0.18$ ). pH and *Cryptosporidium* were not significantly correlated

( $r=0.55$ ;  $p=0.16$ ). Finished water correlations between *Giardia* ( $r=0.25$ ;  $p=0.55$ ) and *Cryptosporidium* ( $r=0.18$ ;  $p=0.66$ ) and pH readings also were insignificant. When only those plants that had protozoa in their finished water were considered, the *Giardia* and pH correlation greatly decreased ( $r=-0.21$ ,  $p=0.79$ ). In contrast, *Cryptosporidium* oocyst and pH correlations were increased ( $r=0.61$ ,  $p=0.38$ ) although this correlation was still insignificant.

### **Effects of Turbidity on Raw Water *Giardia* and *Cryptosporidium* Levels**

Data obtained during this study indicate that turbidity levels in both raw and treated water were not significantly correlated to observed or predicted *Giardia* or *Cryptosporidium* levels (Figure #24-25). Using a correlation analysis, it was, however, demonstrated that raw turbidity levels and *Giardia* cysts show a greater correlation ( $r=0.14$ ;  $p = 0.75$ ) than that seen with *Cryptosporidium* oocysts ( $r=-0.09$ ;  $p=0.82$ ). When only plants demonstrating oocysts in finished water were considered, *Giardia* cysts and turbidity showed a dramatic increase in correlation, although still not significant ( $r=0.78$ ,  $p=0.23$ ). *Cryptosporidium* oocyst and turbidity correlation was still extremely low ( $r=-0.51$ ,  $p=0.49$ ). Correlations between turbidity and protozoan levels in finished water were insignificant (*Giardia*:  $r=0.32$ ,  $p=0.43$ ; *Cryptosporidium*:  $r=0.25$ ,  $p=0.54$ ).



Figure 22 pH Associated with Raw Water Sources From Select Treatment Plants in Eastern Kentucky

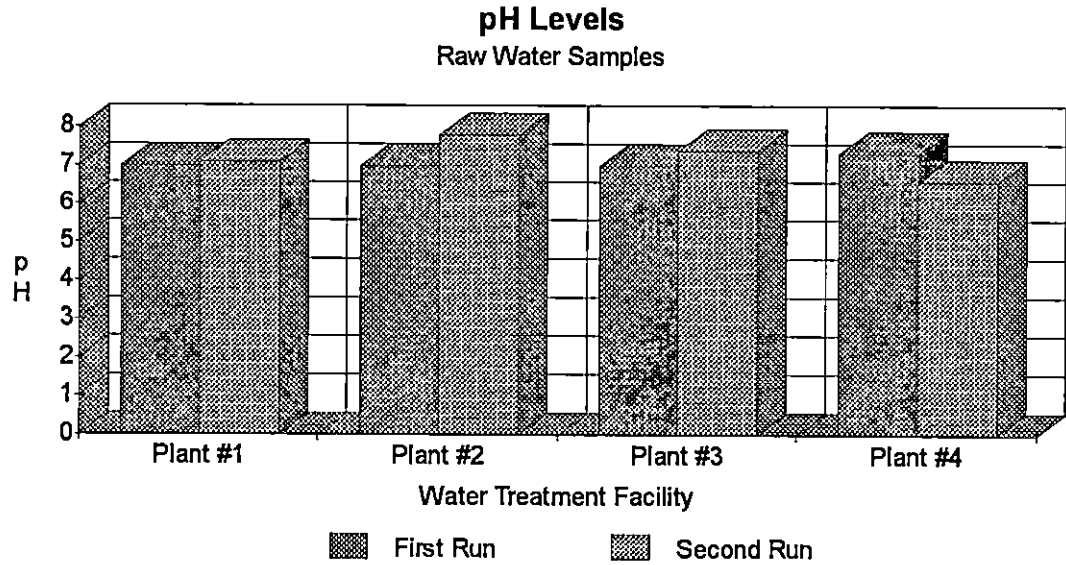


Figure 23 pH Associated With Treated Water Sources From Select Treatment Plants in Eastern Kentucky

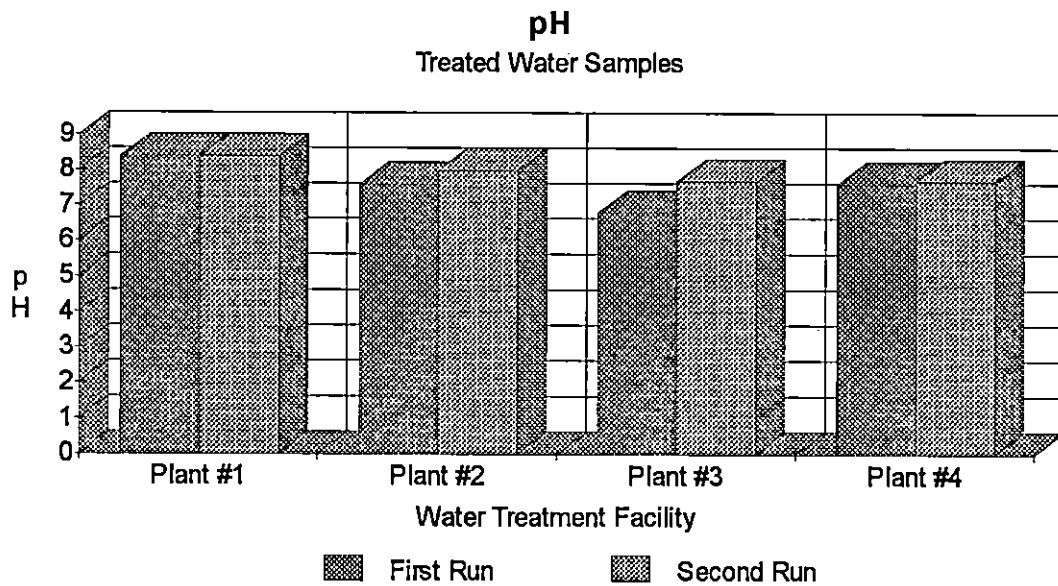


Figure 24 Turbidity Associated with Raw Water Sources From Select Treatment Plants in Eastern Kentucky

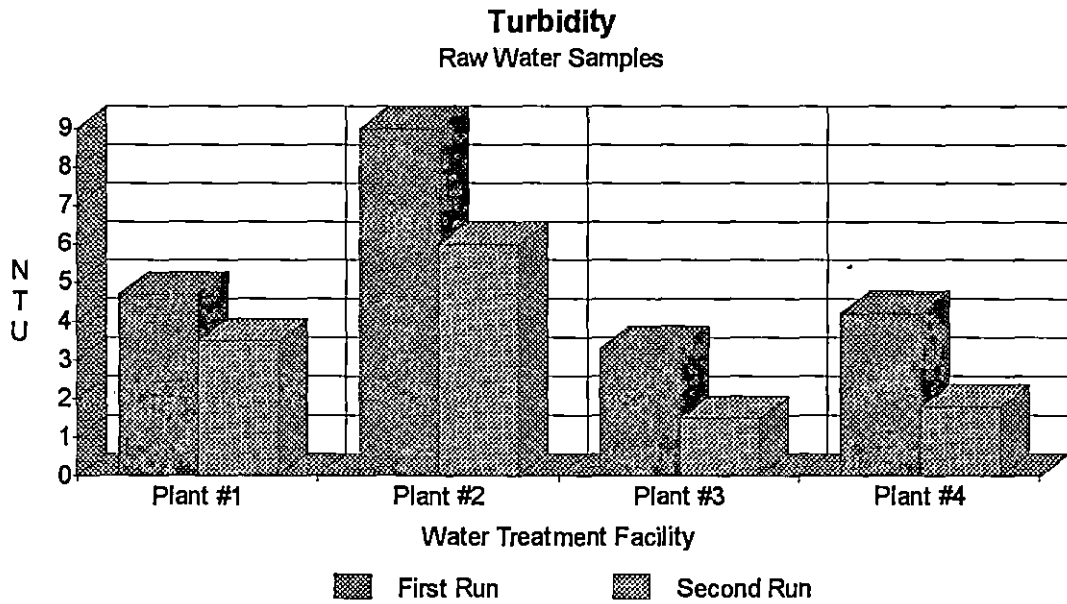
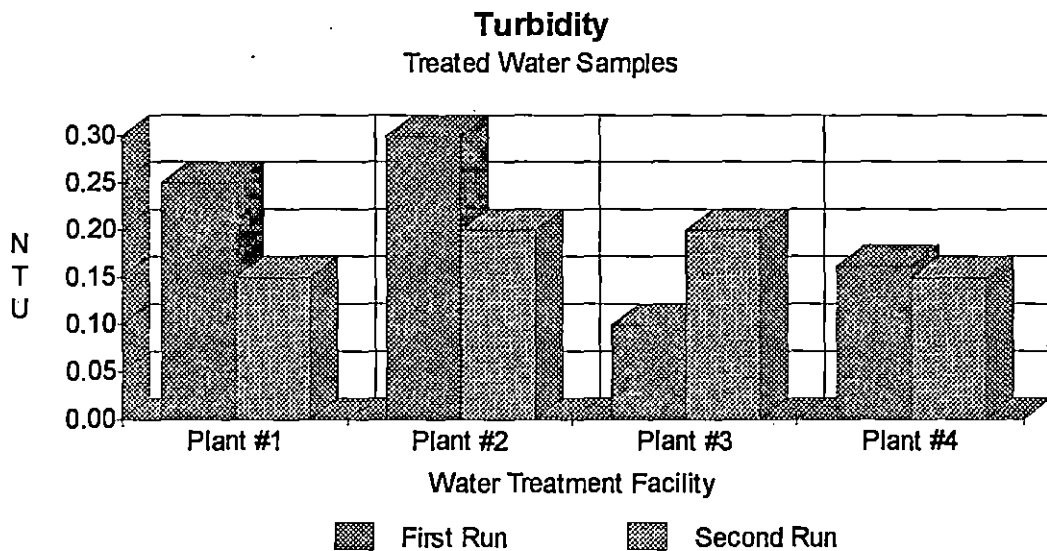


Figure 25 Turbidity Associated with Treated Water Sources From Select Treatment Plants in Eastern Kentucky



### Effects of Fecal Coliforms on *Giardia* and *Cryptosporidium* Levels

Fecal coliforms are commonly used as indicators of fecal contamination. Coliform levels were calculated in order to determine whether or not they would serve as possible surrogate indicators for *Giardia* or *Cryptosporidium* (Figure 26). Fecal coliform levels were not significantly correlated with *Giardia* cysts ( $r=-0.06$ ;  $p=0.89$ ) nor *Cryptosporidium* oocysts ( $r=0.56$ ;  $p=0.15$ ). However, it appears that fecal coliforms may be better indicators of *Giardia* cysts levels ( $p=0.26$ ) than *Cryptosporidium* oocyst levels ( $p=0.59$ ). When only considering those plants positive for pathogenic protozoa in finished water, both cysts and oocysts were poorly correlated with fecal coliforms (cysts:  $r=-0.64$ ,  $p=0.36$ ; oocysts:  $r=0.17$ ,  $p=0.83$ ).

### *Giardia* and *Cryptosporidium* Correlations in Raw Water

A correlation analysis was performed to determine whether or not *Giardia* cysts and *Cryptosporidium* oocysts were correlated in their distribution in raw water sources. Correlation analysis revealed that cysts and oocysts had a very poor correlation ( $r=0.16$ ,  $p=0.7051$ ) at a 95% confidence rate.

### Survey of Treatment Trains for Removal of *Giardia* and *Cryptosporidium*

Several mechanical factors were taken into consideration when testing the quality of filtered water processed by each treatment facility.

Figure 26 Fecal Coliform Levels Associated with Raw Water Sources From Select Treatment Plants in Eastern Kentucky

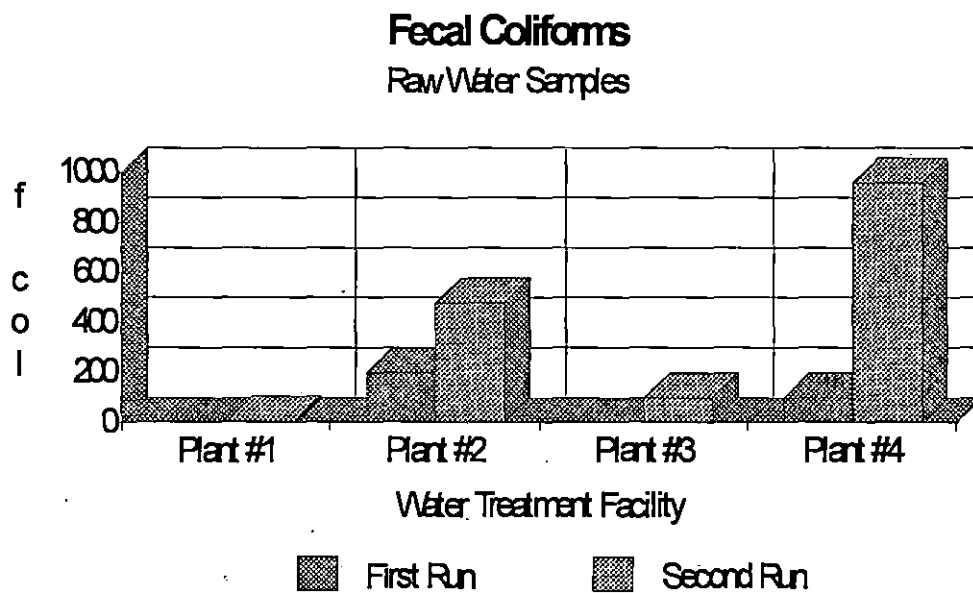


Table 8 Water Quality Indicator Levels Associated With Raw Water Samples From Selected Sites in Eastern Kentucky

Site	Date	pH	Turbidity (NTU)	Fecal Coliforms per 100 mL
Plant #1	5/16/94	7.0	4.7	0
Plant #1	7/6/94	7.1	3.5	6
Plant #2	6/6/94	7.0	9.0	200
Plant #2	7/12/94	7.8	6.0	480
Plant #3	6/14/94	7.0	3.3	0
Plant #3	7/18/94	7.4	1.5	100
Plant #4	9/20/94	7.33	4.2	100
Plant #4	9/26/94	6.6	1.8	960

Table 9 Water Quality Indicator Levels Associated With Finished Water Samples From Selected Sites in Eastern Kentucky

Site	Date	pH	Turbidity (NTU)	Fecal Coliforms per 100 mL
Plant #1	5/16/94	8.4	0.25	0
Plant #1	7/6/94	8.4	0.15	0
Plant #2	6/6/94	7.6	0.30	0
Plant #2	7/12/94	8.0	0.20	0
Plant #3	6/14/94	6.8	0.10	0
Plant #3	7/18/94	7.7	0.20	0
Plant #4	9/20/94	7.62	0.162	0
Plant #4	9/26/94	7.7	0.15	0

Factors considered included filtration method, chemical usage, and chlorine contact time.

Of three treatment trains surveyed, none were shown to be significantly better at removing *Giardia* cysts or *Cryptosporidium* oocysts from filtered water. An analysis of variance procedure revealed that the various treatment trains were not significantly different from each other in their ability to remove pathogenic protozoans from filtered drinking water (n=8, p=0.81 for *Giardia* p=0.85 for *Crypto.*).

A large variation of chemical usage was demonstrated to be common practice at treatment facilities utilized in this study. While chlorine was universally used for disinfection, coagulation and water quality were affected by a broad spectrum of chemicals. Chemical usage was evaluated to see if a particular combination of reagents would lead to lower *Giardia* cysts and *Cryptosporidium* oocysts in finished water. An analysis of variance procedure demonstrated that chemical usage was not a significant contributing factor at lowering *Giardia* and *Cryptosporidium* in filtered drinking water.

Observed chlorine contact times from the treatment facilities did not differ greatly; but, it was of interest to determine if this difference was large enough to significantly affect detected *Giardia* cysts and *Cryptosporidium* oocysts levels. An analysis of variance procedure (p<0.05) revealed that chlorine contact times were not a significant factor in determining cyst and oocyst levels in treated water (p=0.90 for *Giardia* and p=0.87 for *Crypto.*).

Table 10 Chemical Additions Utilized at Select Water Treatment Facilities in Eastern Kentucky

	Plant #1	Plant #2	Plant #3	Plant #4
Potassium Permanganate	X	X		X
Chlorine	X	X	X	X
Alum	X	X		X
Lime	X	X	X	X
Fluoride	X	X	X	X
Soda Ash	X	X		X
Carbon	X			X
Polymer (cat-floc-tl)	X		X	

\*As reported by specified treatment facilities



Table 11 Filtration Methods Utilized at Select Water Treatment Facilities in Eastern Kentucky

Plant	Filter Type	Filter Media	Chlorine Contact (Hrs)
Plant #1	Rapid Sand	Sand Anthracite Garnet	3.5
Plant #2	Rapid Sand	Sand	4.5
Plant #3	Slow Sand	Sand Anthracite	4.5
Plant #4	Direct	Sand Anthracite Garnet	4.78

\*As reported by Treatment Facilities

## Chapter V

### Discussion

*Giardia lamblia* and *Cryptosporidium parvum* were shown to be present in various aquatic environments (two creeks, river, and reservoir) in Eastern Kentucky. These results are consistent with studies showing a national distribution of the protozoa. In 1991, LeChevallier and colleagues surveyed 66 surface water sources from water treatment plants in 14 states and 1 Canadian province. They reported *Giardia* cysts in 81% (69 of 85) of raw water sources surveyed. In contrast, Rose et al. (1991) discovered cysts in 16% (28 of 188) and *Cryptosporidium* oocysts in 55% (102 of 188) surface waters samples from 26 sources in 17 states in the US. Even though a limited number of sources (4) were surveyed, this research determined *Giardia* cysts to be present in 50% (4 of 8) of the raw samples tested in Eastern Kentucky water supplies.

*Cryptosporidium* oocysts were discovered in 63% (5 of 8) of raw water samples tested in Eastern Kentucky being found in three of four sites surveyed. Historically, oocyst levels are found in a greater number of samples than cysts and at higher concentrations. For example, LeChevallier et al. (1991) detected *Cryptosporidium* oocysts in 87% (74 of 85) of raw water samples tested. Rose et al. (1991) observed oocysts in 55% (102 of 188) of surveyed raw water samples. The frequency found in Eastern Kentucky water sources appear to be consistent with those demonstrated throughout the USA.

Various studies appear to show large differences in the levels of cysts or oocysts detected in source waters. A possible reason for these differences is the type and quality of the watershed (Hansen and Ongerth, 1991). Watershed characteristics vary from pristine to those receiving agriculture or sewage runoff. Not surprisingly, both Rose and LeChevallier found *Giardia* and *Cryptosporidium* at higher levels in waters receiving agriculture discharges or sewage effluents than the more pristine watersheds. Watersheds surveyed in this research were primarily classified as residential and agriculture regions. Providing feces from infected livestock or inadequate sewage disposal and straight pipes makes its way into water sources, this could account for the cyst or oocysts contamination.

Analysis of finished water processed by the four Eastern Kentucky water plants revealed the presence of *Cryptosporidium* oocysts in Plant #2 (0.062 oocysts/L) and Plant #4 (0.1206 oocysts/L). Different filtration methods were utilized at each plant: rapid sand filtration (Plant #2) and direct filtration (Plant #4). McFeters (1990) reported that oocysts were found in one of four samples at a plant using rapid sand filtration at concentrations of 0.002 oocysts/L and 0.006 oocysts/L at plants utilizing direct filtration. This data supports the theory that the various filtration methods have similar efficiencies at removing oocysts from raw water. This observation is consistent with data collected in which an analysis of variance procedure determined filtration methods insignificant in predicting oocyst levels in finished water.

*Giardia* cysts and *Cryptosporidium* oocysts were shown not to be correlated in their distribution in raw water sources. This finding contrasts with other studies. For example, Rose et al. (1991) reported a significant correlation of cysts and oocysts in raw water sources ( $r=0.554$ ,  $p<0.001$ ). Our correlation was much lower at  $r = 0.16$ . This difference may again be attributed to the differences in the watershed since every water source has unique factors that influence its overall water quality. In addition, the recovery efficiency of current methods utilized in *Giardia* and *Cryptosporidium* detection range from 9-59%, and may be highly influenced by water quality. This could lead to potentially false negatives or underestimations of levels (McFeters, 1990).

Several standard water quality indicators were analyzed to determine if they could serve as surrogate indicators of *Giardia* or *Cryptosporidium* contamination. Standard procedures utilized in *Giardia* and *Cryptosporidium* analysis are extremely expensive and labor intensive and thus limiting their use by smaller water plants with limited resources. Unfortunately, no standard indicator tested was shown to significantly correlated with cysts or oocysts levels in raw water and thus cannot be used to alert smaller treatment facilities of possible *Giardia* or *Cryptosporidium* contamination.

There are currently two theories on whether or not water quality indicators can alert water plant officials to possible protozoan contamination. Several researchers have reported that their studies reveal a significant correlation between cyst and oocyst concentrations

and water quality indicators such as turbidity and fecal coliforms (Al-Ani et al., 1986; LeChevallier et al., 1991).

Al-Ani et al. (1986) reported that in raw waters having turbidity levels <1 NTU, turbidity was directly proportioned to *Giardia* cysts. Perhaps a drawback of this research is that it was done primarily in low turbidity water. LeChevallier and Norton (1992) also report significant correlations between protozoa and other water quality indicators. They tested source waters which had low, medium, and high turbidity waters. They found that *Giardia* cysts were correlated to fecal coliforms as well as turbidity, pH and total coliforms. *Cryptosporidium* oocysts were shown to be correlated with total coliforms, turbidity, and HPC bacteria.

Rose et al. (1988) also made a comparison to see if cyst and oocyst levels could be significantly correlated to standard water quality indicators. They surveyed two sites at a river in the western United States, the lake outlet and 12 miles down river. Neither bacterial indicator organisms nor turbidity levels were shown to be reliable predictors for the absence or presence of the enteric protozoa. Similarly, Eastern Kentucky sites demonstrated no correlation between cyst/oocyst levels and turbidity or fecal coliforms.

Conflicting data in the field of *Giardia* and *Cryptosporidium* research has prompted the EPA to issue the Information Collection Rule (ICR) (Federal Register, vol. 59, 1994). The purpose of this rule is to conduct a nationwide survey to determine *Giardia* and *Cryptosporidium* levels in surface water. All treatment plants that service more than 10,000 customers will be required to participate in the study. Hopefully,

information collected during this survey will give researchers a better understanding of *Giardia* and *Cryptosporidium* contamination in the nation's surface water and the ability of the treatment plants to effectively remove these protozoans. The data obtained in this study may be helpful to the participating water plants and may allow them to optimize their treatment process.

Turbidity levels demonstrated in this survey were not shown to be correlated with *Giardia* cyst or *Cryptosporidium* oocyst levels. Source waters tested demonstrated fairly low turbidity, 1.5-9.0. These levels are extremely low when compared to levels found by Rose et al., (1988) or LeChevallier and Norton, (1992) which often were 50+ NTU. Thus, the data obtained during this research, in contrast to Al-Ani (1986), seems to suggest that turbidity may not be correlated with *Giardia* or *Cryptosporidium* in low turbidity water.

Much research is now focused on finding a more accurate indicator of *Giardia* and *Cryptosporidium* contamination. Some researchers are suggesting *Clostridium perfringens* as a possible surrogate indicator (Hirata et al., 1981; Payment and Franco, 1993). *Clostridium perfringens* exhibits high survival rate in environmental waters compared to other indicator organisms. Payment and Franco (1993) found that in river samples, only *Clostridium perfringens* was found to be correlated to cyst and oocysts levels.

A colleague determined *Clostridium perfringens* levels for all plants utilized in this study. A correlation analysis revealed an insignificant correlation with *Giardia* cysts ( $r=0.17$ ;  $p=0.69$ ). However,

a much greater correlation between *Clostridium perfringens* and *Cryptosporidium* oocysts was seen ( $r=0.65$ ;  $p=0.08$ ). This correlation bordered on being significant at a 95% confidence level. Although not significant, it was interesting to note that *Clostridium* levels were much higher in raw water sources at those plants that demonstrated oocysts in their finished water. Perhaps with additional sampling, a significant correlation could be demonstrated which would support the results found in other studies.

While much research is aimed at determining which environmental factors most affect *Giardia* and *Cryptosporidium* levels in raw waters, plant treatment trains need to be considered to see if they are the determining factors for permitting the passage of cysts or oocysts into finished waters. Previously, it has been discussed that filtration methods do not seem to affect levels of cysts or oocysts found in treated waters. However, this determination did not consider all the possible chemical treatments used by each plant. Plants studied in this research used a wide array of chemical treatment practices. Data analysis revealed that the combination of chemicals used by each plant did not significantly affect the levels of cyst or oocysts in treated waters. However, it was not determined if individual chemical additions were more important than others at affecting protozoa levels.

Logsdon et al. (1985) evaluated the effects of various filter media and chemical additions commonly used at water treatment facilities. They reported that cyst removal and turbidity removal were enhanced by use of a polymer and high doses of alum, two commonly used

coagulants. Plants surveyed in this study in which oocysts were detected in their finished water did not report the use of a polymer (cat-floc-tl) as part of their normal water purification practices. Those using a polymer as part of their coagulation practices did not have cysts or oocysts in their finished water. Perhaps the use of a polymer affects oocysts levels in treated water in the same fashion as it has reportedly affected *Giardia* cysts levels.

Cysts and oocysts detected in this study were presumptively identified based on size, shape, and fluorescence. Jarroll et al. (1981) have reported that chlorine dosages and contact times play a role in *Giardia lamblia* cyst viability. Some difficulty was experienced in detecting internal structures to confirm identification and viability of cysts or oocysts using DIC microscopy. Perhaps detected cysts or oocysts were empty or too distorted to distinguish. Thus, the chlorine contact time affects on cyst and oocyst viability was not determined due to the limited viewing of internal structures.

When performing analysis for parasitic organisms and possible surrogate indicators, one must keep in mind the extreme variability of watersheds being tested. Current literature gives contrasting views on the reliability of standard indicators, such as pH, turbidity, or fecal coliforms, at predicting *Giardia* or *Cryptosporidium* levels. Studies have also been completed to determine the removal rates and efficiencies of various filtration methods, media, or chlorination practices. In each study, different watersheds are being studied which vary greatly in water quality. Thus, it would be difficult to compare the



removal rates of different filtration methods providing different levels of protozoa existed in initial source waters being tested. One of the goals of the ICR is to develop a better understanding of how environmental factors and treatment schemes influence the levels of *Giardia* and *Cryptosporidium* in drinking water.

This research indicated that *Cryptosporidium* oocysts were present in finished water at Plants #2&#4. Therefore, a determination of risk assessment for cryptosporidiosis is important to consumers receiving drinking water from these filtration plants. Haas (AWWA, WQTC, 1994) ) reported various levels of oocysts that were likely to cause a cryptosporidiosis infection. He stated that 30-50 oocysts/100 L was enough to cause widespread outbreaks; 0.03-30 oocysts/100 L was shown to cause possible individual cases, especially in immunocompromised individuals; while <0.003 oocysts/100 L was considered a low endemic risk level that was unlikely to cause infection. On June 6, 1994, Plant #2 demonstrated 6.2 oocysts/100L in their finished water. This would seem to suggest that cases of cryptosporidiosis are possible depending on the immune status of individual consumers of the finished water. On September 26, 1994, Plant #4 was found to contain 12.06 oocysts/100L. Again, this number falls into the oocyst category range that could possibly cause infection in consumers. It is possible that asymptomatic cases of cryptosporidiosis now exists and goes unreported in consumers of finished water at the before mentioned plants:

Water treatment facilities need to establish a strategy that will enable them to meet the guidelines presented in the Surface Water Treatment Rule in order to lower the risk of a waterborne disease outbreak. McFeters (1990) reported on studies that provided information on how to lower the risk of cysts or oocysts in finished water. They are as follows: 1) use of an adequate coagulant dose, usually 10 mg/L, to attain effective coagulation of suspended particles, 2) maintain a constant low turbidity in finished water (<0.5 NTU) which eliminates the risk of filter disturbances that may dislodge cysts or oocysts from filters allowing passage into finished water, 3) use of alum as a coagulant that is supplement with a cationic polymer which strengthens the floc, and 4) proper filter backwash and maintenance. When all of these practices are utilized at a water treatment facility, the risk of waterborne outbreaks of giardiasis or cryptosporidiosis may be greatly reduced.

If waterborne outbreaks of giardiasis or cryptosporidiosis do occur in a community, water plant officials need to be aware of the steps to take to deal with the outbreak. *Giardia* cysts and *Cryptosporidium* oocysts are environmentally stable but are destroyed by excessive heat. A boil water advisory would need to be issued until the water treatment facility can determine the source of the protozoan contamination and properly deal with the problem.

The data collected during this research project demonstrated that *Giardia lamblia* and *Cryptosporidium parvum* are present in source waters in Eastern Kentucky. Standard water quality indicators were

shown to be inadequate in alerting water plant operators of the possibility of *Giardia* or *Cryptosporidium* contamination. Additional research needs to be undertaken to develop a better understanding of how to ID and eliminate the risk of infection from the pathogenic protozoa, *Giardia lamblia* and *Cryptosporidium parvum*. Also, studies need to be conducted to determine the prevalence of *Giardia* and *Cryptosporidium* in source water utilized by treatment plants in Eastern Kentucky and how source water quality and treatment train efficiency influence the numbers of cysts and oocysts present in finished water.

## Chapter VI

### Conclusions

This study demonstrated that *Giardia lamblia* and *Cryptosporidium parvum* are present in the aquatic environment in Eastern Kentucky. The major conclusions of this research are outlined below:

1. *Giardia lamblia* cysts were shown to be present in source water supplies in Eastern Kentucky.
2. *Cryptosporidium parvum* oocysts were shown to be present in source waters in Eastern Kentucky.
3. *Cryptosporidium parvum* oocysts were shown to be present in finished water supplies treated by select water plants in Eastern Kentucky.
4. Standard water quality indicators (pH, turbidity, and fecal coliforms) do not correlate with observed *Giardia* and *Cryptosporidium* levels.
5. Treatment plant mechanics (chlorine contact time, filtration, or chemical additions) were shown to be insignificant in their affects on observed cysts or oocysts levels in filtered water.

6. *Giardia* cysts and *Cryptosporidium* oocysts were not correlated in their distributions in raw water sources.

## Literature Cited

- Al-Ani, Mohammed Y., David W. Hendricks, Gary S. Logsdon, and Charles P. Hibler. 1986. Removing *Giardia* Cysts From Low Turbidity Waters by Rapid Rate Filtration. *Journal of American Water Works Association* **78**: 66-73.
- Armon, Robert, and Pierre Payment. 1986. A Modified m-CP Medium for Enumerating *Clostridium perfringens* From Water Samples. *Canadian Journal of Microbiology* **34**: 78-79.
- American Water Works Association Satellite Teleconference. 1994. Preventing Waterborne Disease: Is Your System at Risk? Frankfort, April 8.
- Bellamy, William D., Gary P. Silverman, David W. Hendricks, and Gary S. Logsdon. 1985. Removing *Giardia* Cysts With Slow Sand Filtration. *Journal of American Water Works Association* **77**: 52-60.
- Berk, Sharon G., and John H. Gunderson. Wastewater Organisms. A Color Atlas. Boca Raton, Ann Arbor, London, Tokyo: Lewis Publishers, 1993.
- Bisson, J. W., and V. J. Cabelli. 1979. Membrane Filter Enumeration Method for *Clostridium perfringens*. *Applied and Environmental Microbiology* **37**: 55-66.
- Boeck, William C. 1921. The Thermal-Death Point of the Intestinal Protozoan Cysts. *The American Journal of Hygiene* **1**: 365-387.
- Braidech, Thomas E., and Richard J. Karlin. 1985. Causes of a Waterborne Giardiasis Outbreak. *Journal of American Water Works Association* **77**: 48-51.

- Carcia, Lynne S., and David A. Bruckner. Diagnostic Medical Parasitology. pp. 31-39,49-56. Washington: American Society for Microbiology, 1993.
- Clancy, Jennifer, William D. Gollnitz, and Zhanet Tabib. 1994. Commercial Labs: How Accurate Are They? *Journal of American Water Works Association* **86**: 89-97.
- Craun, Gunther F. 1979. Waterborne Giardiasis in the United States: A Review. *American Journal of Public Health* **69**: 817-819.
- Current, William L., Norman C. Reese, John V. Ernst, Wilford S. Bailey, Melvin B. Heyman, and Wilfred M. Weinstein. 1983. Human Cryptosporidiosis in Immunocompetent and Immunodeficient Persons. *The New England Journal of Medicine* **308**: 1252-1257.
- Current, William L., and Thomas B. Haynes. 1984. Complete Development of *Cryptosporidium* in Cell Cultures. *Science* **224**: 603-605.
- Current, William L. 1988. The Biology of *Cryptosporidium*. *American Society for Microbiology News* **54**: 6.5-610.
- Current, William L., and Lynne S. Garcia. 1991. Cryptosporidiosis. *Clinical Microbiology Reviews* **4**: 325-351.
- DeWalle, F. B., J. Engset, and W. Lawrence. 1984. Removal of *Giardia lamblia* Cysts by Drinking Water Treatment Plants. U.S. Environmental Protection Agency, Cinn., Ohio. Pb 84-162-874.
- DeRegnier, Daniel P., Larry Cole, Daniel G. Schupp, and Stanley L. Erlandsen. 1989. Viability of *Giardia* Cysts Suspended in Lake, River, and Tap Water. *Applied and Environmental Microbiology* **55**: 1223-1229.

- Drinking Water Microbiology. Ed. Gordon A. McFeters. New York, Berlin, London, Paris, Tokyo, Heidelberg, Hong Kong: Springer-Verlag, 1990.
- Dykes, Aubert C., Dennis D. Juranek, Rodney A. Lorenz, Susanne Sinclair, Walter Jakubowski, and Robert Davies. 1980. Municipal Waterborne Giardiasis: An Epidemiologic Investigation. *Annals of Internal Medicine* **92**: 165-170.
- E.P.A. 1994. Proposed ICR Method for Detecting *Giardia* Cysts and *Cryptosporidium* Oocysts in Water by a Fluorescent Antibody Procedure. *Federal Register* **59**: 6416-6428.
- Fackelmann, K. A. 1989. Scientists Nab Water-Polluting Parasite. *Science News* **135**: 343.
- Fayer, R., and R. G. Leek. 1984. The Effects of Reducing Conditions, Medium, pH, Temperature, and Time on in Vitro Excystation of *Cryptosporidium*. *Journal of Protozoology* **31**: 567-569.
- Fogel, Doug, Judith Isaac-Renton, Roland Guasparini, William Moorehead, and Jerry Ongerth. 1993. Removing *Giardia* and *Cryptosporidium* by Slow Sand Filtration. *Journal of American Water Works Association* **85**: 77-84.
- Gallaher, Margaret M., Joy L. Herndon, Linda Jean Nims, Charles R. Sterling, Debra J. Grabowski, and Harry F. Hull. 1989. Cryptosporidiosis and Surface Water. *American Journal of Public Health* **79**: 39-42.
- Glicker, Joseph L., and Roger A. Edwards. 1991. Giardiasis Risk From an Unfiltered, Protected Surface Water Source. *Journal of American Water Works Association* **83**: 46-51.
- Graham, Barbara. 1990. A New Bug in Town. *Health* **22**: 38-40.



- Haas, Charles N., Joel C. Hornberger, Uma Anmangandla, Mark Heath, and Joseph G. Jacangelo. 1994. A Volumetric Method for Assessing *Giardia* Inactivation. *Journal of American Water Works Association* **86**: 115-120.
- Haas, Charles. 1994. American Water Works Association Water Quality Technology Conference. San Francisco, November 6-10.
- Hansen, Jahn S., and Jerry E. Ongerth. 1991. Effects of Time and Watershed Characteristics on the Concentration of *Cryptosporidium* Oocysts in River Water. *Applied and Environmental Microbiology* **57**: 2790-2795.
- Hayes, Edward B., Thomas D. Matte, Thomas r. O'Brien, Thomas W. McKinley, Gary S. Logsdon, Joan B. Rose, Beth L. P. Ungar, David M. Word, Paul F. Pinsky, Michael L. Cummings, Margaret A. Wilson, Earl G. Long, Eugene S. Hurwitz, and Denise D. Juranek. 1989. Large Community of Cryptosporidiosis Due to Contamination of a Filtered Public Water Supply. *The New England Journal of Medicine* **320**: 1372-1376.
- Herwaldt, B., G. Craun, S. Stokes, and D. Juranek. 1991. Waterborne Disease Outbreaks: 1989-1990. *Morbidity and Mortality Weekly* **40**: No. SS-3.
- Hirata, T., K. Kawamura, S. Sonoki, K. Hirata, M. Kaneko, and K. Taguchi. 1991. *Clostridium perfringens*, as an Indicator Microorganism for the Evaluation of the Effect of Wastewater and Sludge Treatment Systems. *Water Science & Technology* **24**: 367-372.
- Hunter, Beatrice Trum. 1993. Giardiasis: The Most Common Parasite Infection. *Consumer's Research Magazine* **76**: 8-9.
- Hunter, Beatrice Trum. 1993. *Cryptosporidium*: An Emerging Human Health Hazard. *Consumer's Research Magazine* **76**: 8-9.

- Jakubowski, W., and J. L. Cleasby. 1984. *Giardia* Methods Workshop. pp. 487-498. Proc. AWWA Water Quality Technology Conference. Denver, Co., Dec. 2-5.
- Jakubowski, Walter, Richard Karlin, Gary Logsdon, Robert G. McCall, David W. Hendricks, William Neuman, and Joseph A. Cotruvo. 1985. Waterborne *Giardia*: It's Enough to Make You Sick. *Journal of American Water Works Association* **77**: 14-19,22, 26,84.
- Jakubowski, Walter. 1988. Purple Burps and the Filtration of Drinking Water Supplies. *American Journal of Public Health* **78**: 123-125.
- Jarroll, Edward L., Alan K. Bingham, and Ernest A. Meyer. 1981. Effect of Chlorine on *Giardia lamblia* Cyst Viability. *Applied and Environmental Microbiology* **41**: 483-487.
- Jokipii, Liisa, Suvi Pohjola, and Anssi M. M. Jokipii. 1985. Cryptosporidiosis Associated With Traveling Giardiasis. *Gastroenterology* **89**: 838-842.
- Jokipii, Liisa, and Anssi M. M. Jokipii. 1986. Timing of Symptoms and Oocyst Excretion in Human Cryptosporidiosis. *The New England Journal of Medicine* **315**: 1643-1647.
- Keith, Susan. 1987. Ground Water Quality Protection: State and Local Strategies. *Environment* **29**: 25+.
- Kent, George P., Joel R. Greenspan, Joy L. Herndon, Lynne M. Mofenson, Jo-Ann s. Harris, Thomas R. Eng, and Hetty A. Waskin. 1988. Epidemic Giardiasis Caused by a Contaminated Public Water Supply. *American Journal of Public Health* **78**: 139-143.

- Korich, D. G., J. R. Mead, M. S. Madore, N. A. Sinclair, and C. R. Sterling. 1993. Effects of Ozone, Chlorine Dioxide, Chlorine, and Monochloramine on *Cryptosporidium parvum* Oocyst Viability. *Applied and Environmental Microbiology* **56**: 1423-1428.
- Korich, Dick G., Michael L. Yozwiak, Marilyn M. Marshall, Norval A. Sinclair, and Charles R. Sterling. Development of a Test to Assess *Cryptosporidium parvum* Oocyst Viability: Correlation With Infectivity Potential. Denver: AWWA Research Foundation, 1993.
- Lange, Kelly P., William D. Bellamy, David W. Hendricks, and Gary S. Logsdon. 1986. Diatomaceous Earth Filtration of *Giardia* Cysts and Other Substances. *Journal of American Water Works Association* **78**: 76-84.
- LeChevallier, Mark W., Thomas M. Trok, Michael O. Burns, and Ramon G. Lee. 1990. Comparison of the Zinc Sulfate and Immunofluorescence Techniques for Detecting *Giardia* and *Cryptosporidium*. *Journal of American Water Works Association* **82**: 75-81.
- LeChevallier, Mark W., William D. Norton, and Ramon G. Lee. 1991. Occurrence of *Giardia* and *Cryptosporidium* spp. in Surface Water Supplies. *Applied and Environmental Microbiology* **57**: 2610-2616.
- LeChevallier, Mark W., William D. Norton, and Ramon G. Lee. 1991. *Giardia* and *Cryptosporidium* spp. in Filtered Drinking Water Supplies. *Applied and Environmental Microbiology* **57**: 2617-2621.
- LeChevallier, Mark W., William D. Norton, Ramon G. Lee, and Joan B. Rose. *Giardia* and *Cryptosporidium* in Water Supplies. Denver: AWWA Research Foundation, 1991.

- LeChevallier, Mark W., and William D. Norton. 1992. Examining Relationships Between Particle Counts and *Giardia*, *Cryptosporidium*; and Turbidity. *Journal of American Water Works Association* **84**: 54-60.
- Leland, Dave, Jeremy McAnulty, William Keene, and Gary Stevens. 1993. A Cryptosporidiosis Outbreak in a Filtered Water Supply. *Journal of American Water Works Association* **85**: 34-42.
- Levine, Norman D. 1984. Taxonomy and Review of the Coccidian Genus *Cryptosporidium* (Protozoa, Apicomplexa). *Journal of American Water Works Association* **31**: 94-98.
- Lin, Shun Dar. 1985. *Giardia lamblia* and Water Supply. *Journal of American Water Works Association* **77**: 40-47.
- Logsdon, Gary S., V. Carol Thurman, Edward S. Frindt, and John G. Stoecker. 1985. Evaluating Sedimentation and Various Filter Media for Removal of *Giardia* Cysts. *Journal of American Water Works Association* **77**: 61-66.
- Logsdon, Gary S. 1987. Evaluating Treatment Plants for Particulate Contaminant Removal. *Journal of American Water Works Association* **79**: 82-92.
- Madore, Mary S., and Joan B. Rose, Charles P. Gerba, Michael J. Arrowood, and Charles R. Sterling. 1987. Occurrence of *Cryptosporidium* Oocysts in Sewage Effluents and Selected Surface Waters. *Journal of Parasitology* **73**: 702-705.
- Moore, Gordon T., Michael Cross, David McGuire, Cecil S. Mollohan, Neva N. Gleason, George R. Healy, and Ladene H. Newton. 1969. Epidemic Giardiasis at a Ski Resort. *The New England Journal of Medicine* **281**: 402-407.

- Moore, Anne C., Barbara L. Herwaldt, Gunther F. Craun, Rebecca L. Calderon, Anita K. Highsmith, and Dennis D. Juranek. 1994. Waterborne Disease in the United States, 1991 and 1992. *Journal of American Water Works Association* **84**: 87-99.
- Musial, Cora E., Michael J. Arrowood, Charles R. Sterling, and Charles R. Gerba. 1987. Detection of *Cryptosporidium* in Water by Using Polypropylene Cartridge Filters. *Applied and Environmental Microbiology* **53**: 687-692.
- Nash, J. Madeleine. 1993. The Waterworks Flu. *Time* **141**: 41.
- Ongerth, Jerry E., and Henry H. Stibbs. 1987. Identification of *Cryptosporidium* Oocysts in River Water. *Applied and Environmental Microbiology* **53**: 672-676.
- Patterson, D. J. Free-Living Freshwater Protozoa. A Color Guide. Boca Raton: CRS Press, Inc., 1992.
- Payment, Pierre, and Eduardo Franco. 1993. *Clostridium perfringens* and Somatic Coliphages as Indicator of the Efficiency of Drinking Water Treatment for Viruses and Protozoan Cysts. *Applied and Environmental Microbiology* **59**: 2418-2424.
- Pontius, Fred. 1994. What You Should Know About *Cryptosporidium*. *Opflow* **20**: 5.
- Pontius, Fred. 1994. Part II-What You Should Know About *Cryptosporidium*. *Opflow* **21**: 6.
- Regli, Stig, Joan B. Rose, Charles N. Haas, and Charles P. Gerba. 1991. Modeling the Risk From *Giardia* and Viruses in Drinking Water. *Journal of American Water Works Association* **83**: 76-83.
- Rice, Eugene W., John C. Hoff, and Frank W. Schaffer III. 1982. Inactivation of *Giardia* Cysts by Chlorine. *Applied and Environmental Microbiology* **43**: 250-251.

- Rose, Joan B., Hamid Darbin, and Charles P. Gerba. 1988. Correlations of the Protozoa, *Cryptosporidium* and *Giardia*, With Water Quality Variables in a Watershed. *Water Science and Technology* **20**: 271-276.
- Rose, Joan B., Lee K. Landeen, Kelly R. Riley, and Charles P. Gerba. 1989. Evaluation of Immunofluorescence Techniques for Detection of *Cryptosporidium* Oocysts and *Giardia* Cysts From Environmental Samples. *Applied and Environmental Microbiology* **55**: 3189-3196.
- Rose, Joan B., Charles P. Gerba, and Walter Jakubowski. 1991. Survey of Potable Water Supplies for *Cryptosporidium* and *Giardia*. *Environmental Science & Technology* **25**: 1393-1400.
- Rose, Joan B., Charles N. Haas, and Stig Regli. 1991. Risk Assessment and Control of Waterborne Giardiasis. *American Journal of Public Health* **81**: 709-713.
- Sauch, Judith. 1984. Detection and Identification of *Giardia* Cysts Using Immunofluorescence and Phase-Contrast Microscopy. AWWA Water Quality Technology Conference. Denver, Co.
- Sauch, Judith F. 1985. Use of Immunofluorescence and Phase-Contrast Microscopy for Detection and Identification of *Giardia* Cysts in Water Samples. *Applied and Environmental Microbiology* **50**: 1434-1438.
- Sauch, Judith F., Debbie Flanigan, Marcia L. Galvin, Donald Berman, and Walter Jakubowski. 1991. Propidium Iodine as an Indicator of *Giardia* Cyst Viability. *Applied and Environmental Microbiology* **57**: 3243-3247.
- Smith, H. V., and J. B. Rose. 1990. Waterborne Cryptosporidiosis. *Parasitology Today* **6**: 8-12.

Sobsey, Mark D., Alfred P. Dufour, Charles P. Gerba, Mark W. LeChevallier, and Pierre Payment. 1993. Drinking Water and Health: Balancing Risks. *Journal of American Water Works Association* **85**: 43-48.

Wolfson, John S., James M. Richter, Mary Ann Waldron, David J. Weber, Deborah M. McCarthy, and Cyrus C. Hopkins. 1985. Cryptosporidiosis in Immunocompetent Patients. *The New England Journal of Medicine* **312**: 1278-1282.

## APPENDIX



### Appendix A-1 m-Fc Broth Composition

Type of Constituents	Amount
Dehydrated m-Fc Broth Media	3.8 g
Distilled, deionized water	100 mL
Rosolic Acid	1.0 mL

\*Store at 4 °C

### Appendix A-2 Frasier Reagent Composition

Type of Constituent	Amount
HgCl <sub>2</sub>	5.0 g
HCl	20 mL
Distilled, deionized water	100 mL

\*Bisson and Cabelli, 1979

\*Store at room temperature

### Appendix A-3 Percoll-Sucrose Flotation Medium Composition

Type of Constituent	Amount
Percoll (Sp.Gr. 1.13)	45 mL
Sucrose Solution (2.5 M)	10 mL
Distilled, deionized water	45 mL

\*Store at 4 °C

\*Use within one week of preparation

### Appendix A-4 Sucrose Solution Composition

Type of Constituent	Amount
Sucrose	85.58 g
Prewarmed distilled, deionized water	40 mL
Bring final volume to 100 mL	

\*Store at 4 °C

\*2.5 M

## Appendix A-5 Ethanol-Glycerol Alcohol Series

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95% Ethanol	Glycerol	Reagent Water	Final Volume	Final % Ethanol
10mL	5 mL	80 mL	95 mL	10
20 mL	5 mL	70 mL	95 mL	20
40 mL	5 mL	50 mL	95 mL	40
80 mL	5 mL	10 mL	95 mL	80

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\*Store at room temperature

## Appendix A-6 Eluting Solution Composition

Type of Constituent	Amount / Liter
1% Sodium Dodecyl Sulfate Solution	100 mL
1% Tween 80 Solution	100 mL
1X Phosphate Buffered Saline	100 mL
Sigma Antifoam A	0.1 mL
Distilled, deionized water	700 mL

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\*Use within one week of preparation

\*pH 7.4

\*Store at room temperature

### Appendix A-7 Sodium Dodecyl Sulfate Solution Composition

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Type of Constituent	Amount
Sodium Dodecyl Sulfate	1.0 g
Distilled, deionized water	100 mL

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\*Store at room temperature

### Appendix A-8 Tween 80 Solution Composition

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Type of Constituent	Amount
Polyoxyethylenesorbitan Monooleate 80	1.0 mL
Distilled, deionized water	100 mL

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\*Store at room temperature

Appendix A-9 Phosphate Buffered Saline 10X Stock  
Solution Composition

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Type of Constituent	Amount / Liter
Sodium Chloride	80 g
Potassium Dihydrogen Phosphate	2 g
Hydrated Disodium Hydrogen Phosphate	29 g
Potassium Chloride	2 g
Distilled, deionized water	1 L

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\*Store at 4 °C

Appendix A-10 1X Phosphate Buffered Saline Composition

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Type of Constituent	Amount
10X Phosphate Buffered Saline	10 mL
Distilled, deionized water	90 mL

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\*pH 7.4

### Appendix A-11 Neutral Buffered Formalin Solution Composition

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Type of Constituent	Amount / Liter
Disodium Hydrogen Phosphate	0.762 g
Sodium Dihydrogen Phosphate	0.019 g
Formalin	100 mL
Distilled, deionized water	900 mL

\*Store at room temperature

### Appendix A-12 1% Bovine Serum Albumin Composition

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Type of Constituent	Amount
Bovine Serum Albumin	1.0 g
1X Phosphate Buffered Saline	100 mL

\*Filter through 0.22  $\mu\text{m}$  membrane filter for prolonged storage

\*Store at 4 °C

\*Discard after 6 months

## Appendix A-13 Eriochrome Black T Counterstain Stock Composition

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Type of Constituent	Amount
Eriochrome Black T	0.05 g
Glycerol	5.0 mL
95% Undenatured Ethanol	95.0 mL

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\*Store at room temperature

## Appendix A-14 Eriochrome Black T Counterstain Composition

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Type of Constituent	Amount
Eriochrome Black T Stock Solution	1.0 mL
95% Undenatured Ethanol	95.0 mL
Glycerol	5.0 mL

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\*Store at room temperature

\*Analytical Labs, Inc., Essex Junction, VT