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GIARDIA: HUMAN HEALTH CRITERIA DOCUMENT

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TABLE OF CONTENTS

I. SUMMARY

This document updates information in the U.S. Environmental Protection Agency's (EPA) Drinking Water Criteria Document on *Giardia* (ICAIR, 1984) and is intended to serve as an addendum to that report. Where appropriate, relevant information from the 1984 document is summarized in each chapter of the addendum. For more a more detailed description of information published before 1985, readers are referred to the 1984 document.

This chapter presents a summary of the information contained in Chapters II through VII. Each of these chapters also contain a more extensive summary section. Chapter VIII contains a discussion of research recommendations and Chapter IX lists references.

E. Chapter II. General Information and Properties

Giardia is a protozoan parasite that has been identified as a important cause of waterborne illness. The parasite is transmitted via the fecal-oral route of exposure, and both endemic and epidemic giardiasis can occur. Ingestion of contaminated water is only one source of infection, and the relative importance of waterborne transmission among other risk factors will vary from place to place depending on general sanitation practices. In the United States, contaminated water has caused a number of outbreaks and illnesses but is not likely the most important mode of transmission. *Giardia* is a common cause of illness in travelers and is frequently spread directly from person to person, especially among children or among persons in areas with poor sanitation and hygiene. Although all age groups are affected, the highest incidence is in children. Breast-fed infants under 6 months of age are not likely to be infected.

Large waterborne outbreaks have been reported, and illness has been associated with ingestion of water from unfiltered surface water sources, shallow wells, and during water recreational activities. Contaminated ice used in beverages and food and the person to person

transmission in day-care centers have caused smaller outbreaks. Although infected children in day-care centers are frequently asymptomatic, they may transmit giardiasis to other children, care givers, and family members.

During 1965 to 1996, 133 waterborne outbreaks and almost 28,000 cases of giardiasis have been reported in the United States, primarily in unfiltered surface water systems. *Giardia* has been the most commonly identified pathogen in waterborne outbreaks reported in the United States since 1971. Ten (8%) of these outbreaks were associated with the use of individual drinking water systems or non-potable water sources, and 108 (81%) outbreaks were associated with public water systems; 14 (11%) outbreaks were associated with accidental ingestion of water during recreation. Unfiltered surface water systems were responsible for 56% of the reported waterborne giardiasis outbreaks in the United States. Communities with unfiltered surface water systems have experienced a waterborne outbreak rate that is eight times greater than communities where surface water is both filtered and disinfected. Epidemiological studies of endemic giardiasis have also reported high risks among persons using unfiltered surface water. Based on these data, the 155 million people who continue to use unfiltered surface water in the United States are at a higher risk for waterborne giardiasis than those who drink filtered surface water.

Organisms in the genus *Giardia* are binucleate, flagellated protozoan parasites which exist in trophozoite and cyst forms. While numerous species of *Giardia* have been described in a variety of mammals and in lower vertebrates, there is no general agreement on the criteria which define species in this genus. Criteria used to date include: host specificity; body size and shape, and the morphology of a microtubular organelle, the median body; and biochemical, molecular, and genetic techniques, such as the polymerase chain reaction (PCR) for DNA-based detection and identification. The median body is an organelle that appears to be unique to *Giardia* trophozoites. In this document, *Giardia* responsible for human infections will be found referred to variously as *G. duodenalis*, *G. intestinalis*, or *G. lamblia* reflecting use by those authors cited.

In the *Giardia* life cycle, the trophozoites divide by binary fission, attach to the brush border of the small intestinal epithelium, detach for unknown reasons, then become rounded and elaborate a cyst wall. The viable, environmentally-resistant cyst is excreted in the feces, moves passively through the environment, primarily aquatic, and may be transmitted to another vertebrate host if ingested. Following ingestion, the excystation process is initiated by conditions in the stomach and completed once the excysting trophozoites pass into the less acidic conditions of the small intestine where the trophozoites attach to the small intestinal epithelium. Encystment is initiated by exposure of the trophozoites to bile (exact components unknown) in the upper bowel and continues in the lower small intestine where the trophozoite rounds up and secretes cyst wall components which move into encystment vesicles to begin the process of cyst wall formation.

Recent, carefully controlled studies indicate that cross-species transmission of *Giardia* can occur. Experimental human and animal infection studies offer evidence that rats, mice, dogs, cats, beaver, muskrat, gerbils, and mule deer are capable of harboring *Giardia* that can infect humans. The role of these animals as a source of human infection, however, remains controversial. Of all of these animals, the beaver and muskrat are the most likely candidate mammals that may serve as a source of infection or reservoir of *Giardia* for waterborne outbreaks among humans. Both aquatic mammals can be infected with isolates of *Giardia* from humans, but each has also been shown to harbor strains of *Giardia* that are phenotypically distinct from those found in humans. *G. mictoti*, a species distinct from that in humans, has been found in muskrat. It is possible that the beaver harbors two types of *Giardia*. One type may be highly adapted to this animal and is rarely if ever transmitted to humans. The other type may be one acquired by the beaver from human sources, which can multiply in the beaver and in turn br transmitted via water back to humans. Thus, while *Giardia* that are indistinguishable from those that infect humans are widespread throughout the Animal Kingdom, current evidence remains insufficient regarding their ability to be transferred to humans.

F. Chapter III. Occurrence

4. Prevalence and Waterborne Risks

In the United States, *Giardia* is the most frequently identified etiologic agent causing waterborne outbreaks and the most frequently identified parasite in stool specimens submitted for ova and parasites (4.0% up to 12% depending on the year and state). The prevalence of human infection ranges world-wide between 2 and 5% in industrialized countries and 20 to 40% in developing countries.

High risk groups for giardiasis include infants and young children, travelers to developing countries, the immunocompromised, homosexuals who practice oral-anal intercourse, and persons who consume untreated water from lakes, streams, and shallow wells. Waterborne outbreaks are more common in the United States and Canada than Europe, and this may be due to the larger number of unfiltered surface water systems in North America. Populations in communities with unfiltered surface water or groundwater that has been contaminated by surface water or sewage are at high risk of infection.

Several small foodborne outbreaks of giardiasis have been associated with the contamination of ice and foods by infected food service workers. Restaurant-associated transmission of *Giardia* does not appear to be a significant public health problem. Outbreaks have occurred in day-care populations and prevalence of *Giardia* infection is relatively high in these populations; however, risk factors for the introduction, spread, and persistence of *Giardia* in child day-care centers are not completely understood.

In the United States, waterborne outbreaks of giardiasis have been reported primarily in unfiltered, chlorinated surface water systems. In most outbreaks, disinfection was found to be

inadequate; chlorine concentrations and contact times were insufficient. In a few outbreaks disinfection was interrupted. In many outbreaks neither the turbidity limit nor the coliform limit was exceeded, but outbreaks have also occurred when turbidity levels were increased. Outbreaks have generally occurred in areas of low water temperature where water disinfection may be less effective and *Giardia* cysts can survive for longer periods of time. Outbreaks have occurred in ground water systems emphasizing the need to protect these sources from sewage and surface water contamination. Vulnerable ground water sources that cannot be protected from these sources of contamination should be considered to be at the same high risk of contamination as surface water sources. Filtration may be required for some groundwater sources to reduce waterborne risks. Outbreaks have also occurred in filtered water supplies, and these emphasize the need for proper chemical pretreatment and the importance of good design, installation, maintenance, and operation of treatment facilities. Because 10% of the waterborne outbreaks of giardiasis occurred as a result of distribution system contamination, adequate precautions should also be taken to protect treated water quality during storage and delivery.

Endemic risks of waterborne giardiasis are high among persons who consume untreated water. In the United States, Canada, and New Zealand, endemic risks are higher among populations that use unfiltered surface water compared to those that use filtered surface water.

2. Environmental Occurrence of *Giardia*

Interpretation of occurrence data is dependent upon methods used to detect and quantify the cysts. Methods used to date generally provide little or no information on viability, infectivity, or species identification when *Giardia* cysts are detected in environmental samples. Quantitative data may not be reliable due to low efficiency and precision of methods.

Giardia cysts are distributed worldwide in surface waters, even those of excellent quality. Cysts have been found in surface waters from the Arctic to the tropics. All surface waters

probably always contain *Giardia* cysts at some level. Whether or not they are detected is dependent upon the methods used to collect and analyze water samples. Cyst levels that have been reported generally are on the order of 10^{3-4}/L in raw sewage; 10^{1-2}/L in secondary treated wastewaters, and 10^{0}/L or less in surface waters. Generally, there is no correlation of cyst levels in water with bacterial indicator organisms. Cysts occur in surface waters throughout all months of the year. Occasionally, seasonal variations are reported but these may be site or region specific. When they are reported in North America, the levels are generally higher in the late summer, fall and early winter.

Longitudinal studies using high frequency sampling indicate spikes in cyst levels that might be missed by monitoring programs using low frequency sampling schedules. Cyst levels are generally higher in rivers or streams influenced by agricultural (e.g., cattle or dairy farming) or residential (e.g., sewage outfall) activities. Municipal wastewaters likely always contain *Giardia* cysts at some level.

In the United States, levels of *Giardia* usually reported in water are somewhat lower than *Cryptosporidium* levels. In other countries, e.g., Canada, widespread water surveys have found higher levels of *Giardia* than *Cryptosporidium.*

National, regional, state or local surveys for the occurrence of *Giardia* in water may not be representative of levels for a specific watershed. Sources of contamination and factors affecting the transport and survival of cysts need to be determined for each watershed. It should not be assumed that contamination levels of sources will remain constant. They may fluctuate significantly due to poorly defined factors including weather events, agricultural practices and treatment plant (wastewater and drinking water) infrastructure and operational practices. The first-flush run-off from storm events will significantly affect source water cyst occurrence.

No published reports on the occurrence of *Giardia* in soil or air were found. One study reported the occurrence of cysts on stainless steel and Formica® surfaces in day care centers. Data are sparse to non-existent on quantitative levels of cysts in or on foods. In 26 waterborne outbreaks associated with drinking water, levels of *Giardia* cysts ranging from <1/100L to 580,000/100L were detected from tap or treated water or the water source in unfiltered systems.

The viability and longevity of *Giardia* cysts in the environment is significantly affected by temperature–as the temperature increases, survivability decreases. A small fraction of cysts can withstand a single freeze-thaw cycle. Cysts subjected to repeated freeze-thaws as might occur in the environment are likely inactivated but still will be detected with present methods.

Cyst inactivation in municipal wastewater treatment plant sludge is temperature-dependent. There is a factor or factors in swine manure slurry that results in more rapid degradation of cysts under field conditions. A bacterium that is capable of killing *Giardia* cysts has been isolated from a fresh water stream.

C. Chapter IV. Health Effects in Animals

In animal species (e.g., cats and dogs) whose *Giardia* infections have been studied in detail, the resultant effects resemble those seen in humans. Infected calves also have been observed to have diarrhea and mucus. Mortality appears to be significant in some animals, e.g., chinchillas and budgerigars, but it is rare in humans. *Giardia* infection may occur in animals of any age but is more likely to occur, and to be symptomatic, in young animals. Many, if not most, animals infected with *Giardia* exhibit no symptoms. These animals do, however, serve as sources of infection for other animals. Symptomatic infection in animals that require therapy usually respond to the same agents, with the same caveats, used in treating human infections.

D. Chapter V. Health Effects in Humans

There is a wide clinical spectrum of giardiasis which ranges from asymptomatic infection and acute self-limiting diarrhea to persistent chronic diarrhea, which sometimes fails to respond to therapy. Asymptomatic infection is most common. Symptoms of giardiasis include: diarrhea, steatorrhea, abdominal cramps, bloating, flatulence, pale greasy and malodorous stools, weight loss, and vomiting. Severe disease may result in malabsorption or growth retardation but rarely death. Chronic giardiasis appears to be infrequent, but when it occurs, may persist for years.

As with all diarrheas, fluid replacement is an important aspect of treatment; anti-giardial drugs are also important in the management of the giardiasis. Chemotherapeutic agents used for treatment of giardiasis include metronidazole, tinidazole, quinacrine, furazolidone, albendazole, and ornidazole. Various doses and treatment periods are recommended for each drug. The drugs may have different effectiveness in their ability to clear *Giardia*, and side-effects should be considered. Drug resistance and relapses may occur. Paromomycin has been used to treat giardiasis in pregnant women, but the cure rate may be low.

Progress has been made in understanding the biology of *Giardia*. However, the mechanisms by which *Giardia* produces diarrhea and malabsorption and the key immunologic determinants for clearance of acute infection and development of protective immunity remain poorly understood. Data on the nature of human immune response to giardiasis are somewhat limited, but there are indications that both humoral and cellular responses are present. Most subjects infected with *Giardia* produce detectable levels of anti-parasite antibodies. However, the role of specific antibody to *Giardia* in determining the host's clinical response to infection has not been delineated.

There is variability in the humoral response to *Giardia* infection. Some patients with symptomatic infections fail to develop sufficiently high antibody levels for results to be called positive. In some patients, levels of anti-*Giardia* IgG antibodies remain elevated long after the infection appears to have been eradicated. No sero-diagnostic procedure has been reported that is

capable of distinguishing asymptomatic from symptomatic infection. The presence of anti-*Giardia* antibodies in serum may indicate either past or present infection with *Giardia*, whereas the presence of *Giardia* antigen in stool specimens indicates current infection.

The epidemiology of giardiasis is complicated by an apparent genetic heterogeneity in this species. Differences in virulence, pathogenicity, infectivity, growth, drug sensitivity, and antigenicity have been reported. In endemic areas where extensive heterogeneity exists, mixed infections with more than one genotype may occur.

E. Chapter VI. Risk Assessment

Current risk assessment models have been used to estimate the risk of waterborne *Giardia* infection in the United States. Based on levels of *Giardia* cysts found in treated drinking water in the United States, the annual risks of *Giardia* infection are estimated to be 20 x 10^{-4} (20 waterborne *Giardia* infections per 10,000 persons annually) and may be as high as 250 x 10^{-4} (250 waterborne *Giardia* infections per 10,000 persons annually). These point estimates of risk from drinking water exposures are 10 to 100 times greater than the annual risk suggested that drinking water systems should attempt to maintain (10^{-4} or one waterborne *Giardia* infection per 10,000 persons). However, it is difficult to ascertain the level of accuracy that these risk estimates represent, since no comparable risk estimates are available from epidemiological studies and the risks do not account for viability, speciation, or analytical sensitivity and specificity.

F. Chapter VII. Analysis and Treatment

1. Collection and Analysis of Environmental and Clinical Samples

The absence of a practical cultural method for *Giardia* in environmental samples, and the probability that one could not be developed, led to the development of microscopic examination assay methods. Large-volume sample collection methods were developed using filtration through microporous cartridge media.

Collecting large volume samples of raw source water resulted in many eluates containing a significant amount of particulates that had been retained on the filters. Initially, flotation clarification techniques used zinc sulfate solutions; subsequently, other compounds including sucrose, Percoll, and Percoll-sucrose were evaluated and incorporated into the method. The development of fluorescent antibodies for *Giardia* revolutionized the detection step which had previously been dependent upon examining concentrates with non-selective iodine staining. A combination method was also developed whereby a single sample could be simultaneously assayed for *Giardia* cysts and *Cryptosporidium* oocysts.

The original *Giardia* method was developed to assist in waterborne outbreak investigations. It subsequently was adapted to different applications by those with a need to study drinking water treatment effectiveness, occurrence and distribution of cysts in the environment, or the fate and transport of cysts. In the absence of regulatory requirements to monitor for *Giardia*, there was no official standardized method. However, voluntary efforts through groups such as *Standard Methods for the Examination of Water and Wastewater* and ASTM resulted in consensus reference or proposed methods that could be used as a baseline and modified as needed for particular applications.

The availability of consensus methods resulted in evaluation studies of all steps involved in the methodology including sampling, elution, flotation clarification, and microscopic assay. The sample collection and elution steps were found to account for significant losses of cysts. In addition, aspects of flotation clarification, especially the specific gravity of the gradient solution and the relative centrifugal force used to spin samples, were found to significantly affect

recovery. While retention of cysts and oocysts on the sampling filter was improved by higher turbidities in the water being sampled, the greater quantity of material obtained in the sample pellets presented difficulties in the flotation purification and microscopic assay steps. The nature of the turbidity (e.g., organic or inorganic, particle size, etc.) was more important than the total amount in causing detection and identification problems. For example, algae could make clarification and detection more difficult in certain types of water and at certain times of the year.

The fluorescent antibody assay, while improving detection of cysts, necessitated developing a new definition for identifying cysts. Presumptive cysts were defined by size, shape and apple green fluorescence under specified conditions of reagent type and use and microscope configuration. Confirmed cysts met the presumptive criteria and had defined internal structures characteristic of the genus. These definitions created confusion for interpreting results, especially by persons not familiar with the methodology. Results were often ignored if no confirmed cysts were identified. The presumptive designation included all objects that might be *Giardia* cysts. The confirmed designation was applied to those presumptive cysts that could definitely be identified as *Giardia*. The remaining objects might or might not be *Giardia* because interferences, e.g., cross-reactions or degradation of internal structures are known to occur. Some cysts in a known, purified preparation of *Giardia* will not meet the criteria for confirmation. The presumptive/confirmed terminology was replaced with total counts and counts with internal structures in the *Standard Methods* and Information Collection Rule (ICR) methods. Another limitation of fluorescent antibody identification is that it is only specific to the genus level. While antibodies with various specificities have been developed, the application and interpretation of results with them is complicated by uncertainty in defining species within the genus, and in identifying those species that might have public health significance.

Nucleic acid-based detection and identification techniques have been developed. While they have the potential to specifically detect those species that may be important in human infection and have demonstrated sensitivity down to one cyst in purified preparations, these

techniques have yet to realize their full potential. Problems have been encountered with reproducibility of the assays and with inhibition of the PCR reaction in environmental samples.

The advent of the ICR, and the necessity for developing defined data quality objectives for that monitoring effort, resulted in the collection of performance evaluation data that underscored the low precision of the method in unapproved laboratories. With the promulgation of the ICR, for the first time a process was implemented in the United States for approving and conducting continual performance evaluation of analysts and laboratories that wished to do environmental protozoa analyses. Until that time, adherence to specific methodological protocols, or performance of recommended quality assurance/quality control procedures, was strictly voluntary. Maintaining or developing a similar process after completion of the ICR may help to ensure the reliability of data obtained through continued monitoring efforts.

Increased awareness of method limitations has also spurred development of alternative methods and procedures. In the area of sample collection, sampling 10 L volumes instead of 100 L or more for raw waters is being investigated. Processing the entire concentrate for a 10 L sample may be preferable to processing an undefined portion of a 100 L sample. This may improve the detection limit helping laboratories and drinking water treatment utilities better interpret results. Collecting smaller sample volumes also results in fewer particulates to cause interferences in the detection assay and makes it easier to apply alternate separation technology such as immunomagnetic techniques (instead of flotation separation where cyst recovery is low or erratic). Also, the use of membrane filters with defined porosity (instead of yarn-wound filters with nominal porosities) for sample collection can improve recoveries. For the assay portion of the methodology, much of the tedium and fatigue associated with examining concentrates may be relieved by using techniques such as flow cytometry and cell sorting.

Dependence upon non-cultural methods for the detection and identification of *Giardia* in environmental samples has rendered determining the public health significance of positive

findings problematical. Determining the viability or infectivity potential of small numbers of cysts detected with non-cultural methods has been difficult or impossible to do. A detected cyst may be either viable or non-viable. If the organism is alive, it may be capable of causing infections or, if it has been injured, it may not be infectious. While viability determinations might not be necessary for some applications, such as waterborne outbreak investigations or determining the effectiveness of a treatment process to physically remove cysts, they are very important in assessing disinfection effectiveness and developing risk assessments upon which to base treatment requirements or drinking water regulations.

Procedures used to determine viability have included dye staining, morphological criteria, in vitro excystation, animal infectivity, and nucleic acid-based assays. Traditional dye staining methods (e.g., with eosin) were found not to correlate with in vitro excystation or animal infectivity. Subsequent research produced dyes that enter the viable cyst, e.g., fluorescein diacetate (FDA) and those that are excluded from the viable cyst while they can enter non-viable cysts, e.g.,propidium iodide (PI). Work that has been done with PI to date indicates that cysts stained with this compound are not viable. However, cysts that do not take up the stain may be either viable or non-viable, and whether or not inactivated cysts stain depends in part on how they were inactivated.

At least with *G. muris*, morphological criteria have been shown to correlate with PI staining and animal infectivity. Clearly defined internal characteristics and the absence of a peritrophic space are indicative of non-viable cysts. In vitro excystation also works well with *G. muris* but it is erratic with *G. lamblia* cysts. Another problem is that while excystation may be a good measure of viability for determining disinfectant effectiveness where large numbers of cysts are used in an experimental design, the procedures are not practical for application to the small numbers of cysts likely to be detected in water samples.

Dye staining, morphological criteria and in vitro excystation may be adequate indicators of viability for some applications but they could be conservative in estimating the potential for infection. Animal infectivity has commonly been used in experiments to determine disinfectant efficacy. However, it has seldom been used to evaluate the health significance of environmental *Giardia* isolates because of costs and difficulties with interpreting results from some animal models (i.e., poor specificity).

Nucleic acid-based viability assays have focused on the detection of mRNA by RT-PCR techniques using either the giardin gene or an HSP gene. Amplification of the HSP gene has not proven reliable and there is some question about the survival and longevity of mRNA when the organism is inactivated by different techniques. Besides practical problems relating to the sensitivity and application of PCR techniques to environmental samples, the question of how viability determined by these techniques relates to infectivity remains to be resolved.

For diagnosis of giardiasis in either humans or animals, stools continue to remain the specimen of choice. In humans, the majority of infections can be detected by stool examination, but in some instances, examination of duodenal or intestinal fluids (by aspiration, biopsy or string test) or the use of radiological procedures may be necessary. Fresh stools can be used to prepare wet mounts that are examined by conventional light microscopy for the presence of cysts or trophozoites.

Fresh, frozen or preserved stools can be examined using traditional dye staining techniques or with increasingly popular immunofluorescence assays. A variety of commercially-available fluorescent antibody kits that target cysts or antigens are available. Evaluation of these kits indicates that they have a high degree of sensitivity and specificity. They may require less time to perform and produce results with a single stool sample equivalent to fresh stool and dye staining techniques that require multiple stool examinations. The use of flow cytometry with

immunofluorescence reagents may allow a greater number of human or animal specimens to be examined in a given time period with less operator fatigue.

For surveys of giardiasis in animal populations, examination of intestinal scrapings from live-trapped animals may prove more fruitful than examination of feces from kill-trapped animals. With either human or animal specimens that have been frozen and thawed before examination, immunofluorescence assays are more likely to detect cysts than is examination by conventional microscopy. This may allow samples to be archived and subsequently re-examined for a variety of purposes, including quality control. One author concluded that phase microscopy had an advantage over immunofluorescence assays in that phase microscopy allowed some differentiation to the species level of cysts found in wild rodent populations.

Sero-diagnosis is still not a useful technique in the clinical setting due to the inability to distinguish between present and prior infections. However, serologic testing may have value in conducting epidemiological studies. Secretory antibody has been detected in a small study of saliva specimens from patients infected with *Giardia*, but the potential for developing tests that could be useful for either diagnostic or epidemiologic purposes remains to be determined. Also, the development and application of gene probe techniques (e.g., PCR) for clinical diagnostic purposes has thus far proved challenging due to inhibitory substances in feces and resulting problems with sensitivity and specificity.

5. Water Treatment

Information obtained during the past 20 years from laboratory, pilot plant, and full scale treatment plant studies show that *Giardia* cysts can be effectively removed and inactivated by a combination of filtration and disinfection. Because of the low infectious dose for *Giardia*, the wide-spread occurrence of the infection in humans and a variety of animals, and the relative resistance of *Giardia* cysts to environmental conditions and water disinfectants, it is important to

consider multiple barriers for the protection and treatment of both surface and ground water sources: a combination of watershed protection for surface waters, well-head and aquifer protection for ground water sources, water filtration, disinfection, and protection of the integrity of the distribution system. Use of all of these barriers affords the most effective means for assuring the microbial safety of public water supplies.

It is impossible and morally unacceptable to eliminate wild animals from a watershed, but their affect on source water quality can be reduced. The strict control of contamination from farming, domestic animals, and human sewage discharges can also reduce contamination of source waters. Wells and springs should be protected from the influence of surface water and sewage discharges from septic tanks and municipal wastewaters. While watershed management practices can reduce the potential for contamination, they cannot eliminate it. To effectively protect against the waterborne transmission of *Giardia*, adequate water treatment is also required. For surface water sources and groundwater sources under the influence of surface water, both disinfection and filtration are recommended. Filtration exceptions may be granted where water sources meet criteria of EPA's Surface Water Treatment Rule (SWTR); however, if water sources are also subject to contamination with *Cryptosporidium*, it should be remembered that disinfection levels used to inactivate *Giardia* cysts may not be sufficient to inactivate *Cryptosporidium* oocysts.

Filtration technologies commonly used by water supplies can be designed and operated to remove 99% or more of *Giardia* cysts. Conventional and direct filtration, when operated under appropriate coagulation conditions, can remove 99.9% to 99.99% of *Giardia* cysts. The highest removal rates occurred in pilot plants and water utilities that optimized coagulation and achieved very low finished water turbidities (0.1- 0.3 nephelometric turbidity units). Cyst removal was poor in filtration plants where coagulation was not optimized even though the turbidity of filtered water was low.

Conventional and direct filtration facilities should include chemical pretreatment to provide adequate coagulation. In some source waters, sedimentation may be needed to effectively remove cysts. Removals similar to or better than conventional and direct filtration effectiveness have been found for slow-sand and diatomaceous earth filtration, but operational and other factors are important in maintaining high removals of cysts by these filters. Low water temperatures may adversely affect the efficiency of slow sand filters.

Membrane filtration is promising for some water systems, but care must be exercised when selecting the type and effective size of the membrane. If *Giardia* cyst removal is desired, the effective size of the membrane should be rated to remove at least 99.9% of cysts or cyst-sized particles. It should be remembered that membranes that are effective for removing *Giardia* cysts may not be effective for removing other protozoa of a smaller size, such as *Cryptosporidium*, *Cyclospora*, or microsporidia. High levels of cysts are found in filtered backwash water, and this potential source of contamination should be considered before this water is discharged to the environment or recycled back to the beginning of the water treatment plant.

Disinfectants can also achieve 99% or greater inactivation of *Giardia* cysts, but the effectiveness of a chemical disinfectant may be affected by factors including water temperature and pH, applied and residual disinfectant concentration and contact time, particles which may shield cysts from contact with the disinfectant, and organic matter which may cause disinfectant demand. Filtration can make disinfection more effective by reducing the disinfectant demand and removing particles that may interfere with disinfection effectiveness.

The EPA regulates disinfectants and disinfection by-products, and this limits the concentration and contact time of any chemical disinfectant that can be applied. When lower concentrations of a disinfectant are required to meet disinfection and disinfection by-product limits, both filtration and disinfection may be necessary.

Disinfection employed by the water industry can inactivate *Giardia* cysts; however, cysts can be resistant to low doses of chlorine and chloramines, and there are differences between the inactivation efficiencies of the various disinfectants. The reported effectiveness of inactivation by the typically utilized water disinfectants, in decreasing order of efficiency, is as follows: ozone, mixed oxidants, chlorine dioxide, iodine, free chlorine, and chloramines. Under current operating conditions and with current designs, ultraviolet irradiation does not appear to be useful for disinfection of *Giardia* cysts. *Ct* (disinfectant concentration and contact time) values are available to compare disinfectants, and values are recommended for various conditions of water temperature and pH. Applied and residual concentrations, as well as how the disinfectant is applied, are important to consider. For example, ozone-peroxide is less effective than ozone and preformed chloramines are less effective than chloramines that are not preformed. Since *Ct* values are based on results of laboratory studies in demand-free water, caution is recommended in extrapolating these data to natural waters and beyond the experimental conditions. If source waters are heavily contaminated with *Giardia* cysts, disinfection alone may not be sufficient to protect against waterborne infection. Even though disinfection is adequate to inactivate 99.9% of *Giardia* cysts in heavily contaminated source waters, sufficient numbers of cysts may survive to cause infection in a fraction of the population.

II. GENERAL INFORMATION AND PROPERTIES

B. History and Taxonomy

Organisms in the genus *Giardia* are protozoa that are parasitic in the intestinal tract of humans and a wide variety of vertebrates. Dobell (1932) persuasively argued that *Giardia* was the first protozoan to be described; the description was recorded by Antony van Leeuwenhoek in 1681 after examining his own diarrheic stool with one of his simple microscopes.

The available evidence supports the idea that *Giardia* can cause human intestinal disease as well as disease in some lower animals. *Giardia* has not always been considered a human pathogen because asymptomatic human infections are common. If exposure to an organism fails to consistently result in symptoms or disease, it is difficult to satisfy Koch's postulates which define the causal relationship between a microorganism and a specific disease (Last, 1995). Until the second quarter of this century, most physicians believed *Giardia* to be a harmless intestinal commensal, primarily because the parasite was identified in many persons who did not have symptoms of disease.

The introduction of the drug quinacrine as a means of eliminating *Giardia* infection played a role in the recognition of *Giardia* as a pathogen. The administration of quinacrine resulted in the simultaneous disappearance of these protozoa as well as the host's intestinal symptoms, lending credence to the pathogenic nature of the *Giardia* (Brumpt, 1937; Galli-Valerio, 1937). A more recent study of infection in human volunteers confirms Koch's postulates for *Giardia* (Nash et al, 1987).

We now know that this genus contains a variety of related organisms parasitic in the intestinal tract of many vertebrate species. While historically, various genus names have been applied, *Giardia* is now widely accepted as the genus name for this group. How to designate

species in this genus has been a question debated for at least a half century. Until the 1950s, it was common to assign *Giardia* species names on the basis of a belief in strict host specificity and the importance of protozoan body dimensions. This resulted in more than 40 *Giardia* "species" being described (ICAIR, 1984). We now know that *Giardia* dimensions are not a reliable sole criterion for *Giardia* speciation and that all *Giardia* are not strictly host specific. Thus, the earlier species criteria have been called into serious question. While recent enzyme, DNA, RNA and morphologic studies have provided data important to the resolution of this question, there is no general agreement as to *Giardia* species criteria.

Tibayrenc (1994), in considering whether *Giardia* is a complex of several species or not, concluded: "Due to the fact that *Giardia* is probably a clonal organism, the biological concept of species cannot be used to address the question." Reflecting the opinions of the contributors to the scientific literature, the *Giardia* responsible for human infections will be found in this document referred to variously as *G. duodenalis*, *G. intestinalis*, or *G. lamblia*.

B. Life Cycle of *Giardia*

Information on the life cycle of *Giardia* is important for characterizing the two stages of the genus, and the steps which occur when the organism changes from one stage to the other. In addition, information on the life cycle is of interest in understanding the health effects, transmission of the cysts, development of symptoms of giardiasis, and identification of the methods of in vitro cultivation to study the survival of the stages, and for producing cysts for conducting experimental studies. The life cycle of *Giardia* was described in the previous document (ICAIR, 1984) and recently reviewed by Marshall et al. (1997).

Giardia have a simple life cycle (ICAIR, 1984) in which the flagellated, binucleate trophozoites reside in the upper two thirds of the host's small intestine (the duodenum and jejunum), where they attach, by means of a ventral adhesive disk, to the brush border of the

epithelium, and reproduce by binary fission. The trophozoite-to-cyst transformation takes place in the intestinal tract except in cases where the encystment process may not be completed within the intestines of the host. Sometimes the transition from trophozoite to cyst fails to be initiated within the small intestine, or to be completed following transit through the intestinal tract and excretion with the feces (Schaefer, 1990). This may happen in situations when there is rapid intestinal transit and the residence time is not sufficient for cyst formation, especially in cases of severe diarrhea. Thus, diarrheic stools from patients with giardiasis may frequently contain trophozoites (ICAIR, 1984). The life cycle continues with excretion of the cyst, followed by the subsequent ingestion of the cyst by a suitable vertebrate host (ICAIR, 1984).

1. Excystation

The passage of viable cysts through the stomach initiates the process of excystation, whereby the trophozoite emerges from the encysted stage. Excystation is completed in the small intestine (ICAIR, 1984). At the time of excystation, a quadrinucleate trophozoite in the process of division emerges from the cyst wall, and promptly completes the division process, yielding two binucleate trophozoites (Bingham and Meyer, 1979).

a. In vivo Excystation

Gillin et al. (1988) presented information on the conditions for in vivo excystation of *Giardia* cysts. It is crucial to the life cycle that the cysts not excyst and trophozoites emerge in the stomach, because the trophozoites would be killed by the gastric acids. These stomach acids and the resulting low pH play a role in triggering excystation, but the trophozoites will not emerge until a neutral pH is encountered in the upper portions of the small intestine.

b. In vitro Excystation

The in vitro excystation of *Giardia* cysts from human feces was successfully induced by a combination of filtration and purification, employing centrifugation and layering of the cysts on 0.85 M sucrose (ICAIR, 1984). Exposure of purified cysts to synthetic gastric juices at pH 1.6 induced excystation to occur within 30 minutes. When the individual components of the gastric juices were tested, only the hydrochloric acid (HCl) was required to induce excystation. Because exposure of *Giardia* cysts to variety of acids at pH 2.0 induced significantly higher percentages of excystation than water controls, it was concluded it is the hydrogen ion, rather than the specific counter ion, was necessary to induce excystation of *Giardia* (ICAIR, 1984).

Other techniques have been developed to improve both the cyst purification and excystation methods. Sauch (1984) reported that a Percoll solution at pH 7 and 1.08 g/cm^3 gave consistently higher cyst recoveries than either the sucrose or zinc sulfate methods. To improve the purification of the recovered cysts, cyst suspensions were centrifuged at different sedimentation velocities at unit gravity in Percoll density gradients from 1.01 to 1.03 g/cm^3. Consistently high levels of *Giardia* excystation ranging from 40 to 95% were found with a procedure that requires a low-pH induction step (involving three separate solutions) and an excystation step (ICAIR, 1984). Sauch (1988) conducted excystation trials with a modification of the previous procedure. Trypsin, serum, or bile salts were replaced by peptone, and excystation was observed in most tests, indicating that neither trypsin, serum, nor bile salts is required for excystation. However, the mean percentage of observed excystation ranged from 1% to 96%, indicating there were sources of substantial variability that remained to be identified.

Schaefer (1990) reviewed studies which described the excystation process. In the process, the caudal flagella and distal ends of the other flagella extend outside the cyst wall and begin to move slowly. Within 5 to 10 minutes of emergence, the flagella beat rapidly pulling and/or breaking the trophozoite out through the cyst wall. This appears to result in a tearing of the cyst wall, which further facilitates the release of the trophozoite from the cyst.

2. Encystation

Encystation is the process whereby the trophozoite transforms into the cyst stage of the life cycle. During the intestinal portion of the *Giardia* life cycle, some trophozoites detach from the brush border for various reasons and enter the fecal stream. The process of encystment begins in the small intestine with the trophozoites becoming rounded and elaborating a cyst wall; the resultant cysts are then excreted with the feces (ICAIR, 1984).

a. In vivo Encystation

Danciger and Lopez (1975) observed three patterns of *Giardia* cyst excretion or production in 15 infected children over a 1-to 3-month period. In children labeled as "high cyst excreters," large numbers of cysts were present in nearly all stool specimens. In contrast, "low cyst excreters" had detectable levels of cysts in only 40% of the stool specimens. A third group, "mixed excreters," had 1-to 3-week periods of high cyst excretion, alternating with periods of low cyst excretion. No correlation was found between the numbers of cysts produced and the consistency of the stool or frequency of defecation. Furthermore, the use of purgatives failed to increase cyst production.

Grant and Woo (1979) observed cyclic cyst excretion by laboratory rats and mice experimentally infected with *Giardia simoni* and *Giardia muris*, respectively. This cyclic cyst excretion exhibited periods of 7-8 days between peaks and was also observed in captive deer mice and meadow voles that had been naturally infected with *Giardia*. Craft (1982) reported that when rats were infected with *G. lamblia* from humans, they exhibited a similar cyst excretion pattern to that observed in humans by Danciger and Lopez -- 12% of the rats excreted large numbers of cysts continuously, 80% excreted variable numbers of cysts intermittently, and no cysts were excreted by 8% of the infected rats.

b. In vitro Encystation

Gillin et al. (1987) conducted in vitro studies of the encystation and expression of cyst antigens by *G. lamblia*. Cultured trophozoites tested in the presence of bile salts showed the importance of bile salts to the process of encystation. Exposure to primary bile salts yielded greater levels of encystation than exposures to secondary bile salts. In addition, the cultured trophozoites exhibited more than a 20-fold increase in the numbers of oval, refractile cells that reacted strongly to anti-cyst antibodies. The refractile cells also showed higher levels of expression of major cyst antigens.

Schupp et al. (1988) described the morphology of *Giardia* encystation in vitro. In several strains grown axenically, light microscopy examination revealed an identical morphology with *Giardia* cysts isolated from fecal samples. These morphological comparisons were based on characteristic size and shape of the cysts and the presence of 2 to 4 nuclei. The cysts grown in vitro were found to exhibit a similar positive immunoreactivity for the cyst wall.

The conditions required to trigger the encystment process in vitro have also been described in detail. Gillin et al. (1989) characterized the roles of bile, lactic acid, and pH in the completion of the life cycle of *G. lamblia* under in vitro test conditions. Bile and alkaline conditions, such as those found in the lower small intestine, induced high levels of encystation. In addition, lactic acid, which is a major product of bacterial metabolism within the colon, was found to have stimulated the encystation process. The cysts produced in these tests exhibited greater than 90% viability based on uptake of fluorogenic dyes and exclusion of propidium iodide (PI), two conditions associated with the viability determination. See Section VII., Chapter VII for a discussion of the studies concerning cyst viability. Gillin et al. (1989) reported that this was the first quantitative demonstration of the in vitro complete life cycle for *G. lamblia*.

Campbell and Faubert (1994) evaluated both the in vitro and in vivo encystation of *G. lamblia*. Examinations of the intestines of the gerbil indicated that trophozoites and encysting trophozoites were found in the three equal sections of the small intestine; much lower numbers were found in the colon. Conversely, cysts were only found in the lower two sections of the small intestine and the colon. In the in vitro tests with four strains of *Giardia*, there were significant differences in the production of encysting trophozoites and cysts, but in vivo tests in gerbils did not reveal similar differences. The encysting trophozoites were characterized as having the presence of encystation-specific vesicles (ESV), and there may be cyst antigens. Campbell and Faubert (1994) concluded that these encysting trophozoites represent a transient population of cells which appeared during both in vitro and in vivo encystation, that the relative differences observed in encystation among the strains during in vitro testing were not reflected in vivo, and that passage through the gerbil during one cycle of encystation/excystation can result in disparate test results during in vitro encystation testing.

Erlandsen et al. (1990a) analyzed for the presence of these cyst wall antigens by field emission scanning electron microscopy, and located their presence in the filaments associated with the outer portions of intact cysts and on the developing cyst wall filaments in encysting trophozoites. With polyclonal and monoclonal antibodies specific for the cyst wall antigens, there was strong labeling observed on the filamentous cyst wall, but no labeling on the membranous portion.

McCaffery and Gillin (1994) conducted studies on protein transport during the processes of growth and encystation. The endomembrane system has proteins present during growth and encystation, but the ESV are novel secretory vesicles, that serve to transport cyst antigens to the nascent encysting wall. These results suggest that *Giardia,* which is a primitive parasite, has evolved various complex structures for protein transport to the cell wall.

McCaffery et al. (1994) further studied these protein transport mechanisms. There is a progression of the types of antigenic chemicals in these vesicles, and that there may be indications of the initiation of a stage in the antigenic switching which is differentiation-driven. At this stage, while the cyst wall is being laid down, the antigen might no longer be produced or transported to the wall, but may be taken back into the cell. This process might facilitate immune evasion by the *Giardia*, both by providing a covering over the trophozoite surface and by initiating the antigenic switching, which appears to provide the trophozoite with increased resistance to host-mediated defenses.

C. Morphological Features

Members of the genus *Giardia* are flagellated protozoan parasites belonging to the phylum Sarcomastigophora, class Zoomastigophorasida, order Diplomonadida, and family Hexamitidae. All organisms in this genus are parasites which occur in trophozoite and cyst forms (ICAIR, 1984).

The parasite adaptations promoting cyst survival in the external environment, and trophozoite infectiveness and persistence in the mammalian small intestine, each contribute to being key virulence properties for this parasite to cause symptomatic disease (Aley and Gillin, 1995). However, the actual properties of the trophozoites that cause the diarrhea, such as toxins or conventional virulence factors (if such exist), have not yet been identified.

The application of modem biochemical techniques has resulted in rapid advances in our understanding of a number of areas of *Giardia* metabolism (Mendis and Schofield, 1994; Paget et al, 1989, 1993). These studies have shown that the metabolism of these organisms is far more complex, and that they have the ability to use a far wider range of substrates, than was originally believed.

1. Trophozoite

Trophozoites of the genus *Giardia* inhabit the upper small intestine of the vertebrate host (ICAIR, 1984). According to Meloni et al. (1995), the trophozoites are vegetative in that they are "capable of growing" and "function in processes such as growth and nutrition and not in sexual reproduction." *Giardia* appear to reproduce only asexually; sexual reproduction has not yet been reported for this protozoan genus.

The trophozoites are parasitic on the wall of the small intestine, but the pathophysiology of infection by the trophozoites is poorly understood. The major anatomic change being blunting of the villi in the small intestine. The trophozoites are not invasive of the epithelial cell layer and can survive only within the small intestine (Aley and Gillin, 1995).

The pyriform bodies of trophozoites of the genus *Giardia* range from 9 to 21 µm long, 5 to 15 µm wide, and 2 to 4 µm thick (ICAIR, 1984). Trophozoites are identified by the presence of two morphologically indistinguishable anterior nuclei, eight flagella, two central axonemes, microtubular median bodies, and a ventral adhesive disk. A pair of staining structures (median bodies) lie dorsal to the axonemes and are tipped dorsoventrally and anterioposteriorly so that the right tip is more dorsal and anterior (ICAIR, 1984). These are found in every species of *Giardia* described. Median bodies consist of random arrangements of microtubules that lack an origin or insertion into any other structure and may play a supporting function in the posterior portion of the trophozoite behind the striated (ventral) disk (ICAIR, 1984).

2. Cyst

Giardia cysts are typically ovoid, and measure from 10 to 15 µm in length, and from 7 to 10 µm in width, with the cyst wall being approximately 0.3 µm thick (ICAIR, 1984). Newly

formed cysts contain two morphologically indistinguishable nuclei. Each nucleus in the cyst undergoes a single further division, so that mature cysts contain four nuclei.

Filice (1952) stated that the median bodies of the trophozoites were rarely, if ever, seen in cysts, but Sheffield and Bjorvatn (1977) found a group of randomly arranged microtubules near the flagellar axonemes in cysts that could be median bodies. They also observed that the microtubules were less compact than those observed by Friend (1966) in the trophozoite, possibly accounting for the apparent absence of median bodies in cysts when viewed with visible-light microscopy. Gillin et al. (1989) reported that a median body is visible in what they designate as Type I cyst when viewed in relief with Nomarski differential interference contrast optics. Gillin et al. (1989) described these Type I *G. lamblia* cysts as water resistant, oval shaped, smooth, and refractile, with cyst wall, axostyle, and median body visible in relief by Nomarski differential interference contrast optics.

Jarroll et al. (1989) demonstrated that a substantial component of the purified cyst wall (PCW) is comprised of a polymer of galactosamine or N-acetylgalactosamine, with a structure as yet undescribed, but with a function comparable to chitin, providing both physical strength and resistance to chemicals. These polysaccharides and putative proteins of the PCW may also play a role in excystment, functioning in some way in signal transduction and recognizing the proper chemical stimuli leading to the excystment of the cyst and release of trophozoites, following passage through the host stomach and when entering the small intestine (Aley and Gillin, 1995).

Cysts, unlike the trophozoites, are not motile, and must be protected from wide variations of pH, temperature, and osmolarity. Cysts also provide protection from hypotonic lysis, since it has been observed that trophozoites, which are shed during extreme cases of diarrhea without going through encystment, will readily disintegrate. One of the document authors (Meyer) found that when laboratory cultured *G. lamblia* trophozoites are removed from culture medium and placed in tap water, the trophozoites become enlarged and undergo lysis within 30 minutes.

These results are consistent with the fact that no osmoregulatory systems have been reported in the trophozoites of these or other cyst-forming parasitic protozoa.

On the other hand, *Giardia* cysts may remain viable in water for long periods of time under typical environmental conditions. For example, after being stored in water for 77 days at 8 C, the encysted forms were found to be viable by dye testing (Bingham et al., 1979). The encysted forms were also found to be capable of excystation in mouse infectivity tests after periods of 28 days storage in water with longer survivals observed at lower temperatures of less than 10 C (deRegnier et al., 1989). See Section H.3., Chapter III for further details of these studies.

The metabolism of *Giardia* cysts has been studied. Paget et al (1989, 1993) have compared oxygen uptake in *Giardia* cysts and trophozoites from both human and mouse sources. Since the oxygen uptake of cysts has been shown to be 10 to 20 percent of that of trophozoites, one can conclude that these protozoan forms are not dormant in the sense that bacterial endospores are. Rather, *Giardia* cysts continue to metabolize, but at a rate that is much less than that of their trophozoite counterparts.

D. Species Transmission

1. Direct Transmission Between Humans

Early studies demonstrated that *Giardia* cysts, ingested either in capsules or in water, were capable of excysting and proliferating in the challenged host; most infections disappeared spontaneously, different patterns of infection were observed, depending on the donor source of cysts, and some infected persons failed to shed cysts for a long period (ICAIR, 1984). See Section A, Chapter VI for further discussions of these studies. More recently, Nash et al.(1987) reported successful infection of human volunteers from inoculations of cultured *Giardia*

trophozoites that had been isolated from two infected persons. Five volunteers each received 50,000 trophozoites of one isolate; another group of five each received 50,000 trophozoites of the other cultured isolate. All volunteers in the group receiving the first isolate became infected; three developed symptoms of giardiasis. None of the five volunteers in the group receiving the second isolate became infected. This result is consistent with earlier observation (ICAIR, 1984) that strain differences may exist between *Giardia* isolated from different human hosts, and it suggests that failure of an isolate to cause infection in a particular host is insufficient evidence for assuming that it cannot be infective for that species host. Nash et al. (1987) proposed that these results fulfilled Koch's postulates for *Giardia*.

2. Transmission Between Animals and Humans

Research on the cross-species transmission of *Giardia* is important to identify sources and reservoirs of infection. That is, can human-isolated cysts cause infection in animals and can animal-isolated cysts cause giardiasis among humans? It is difficult to interpret the results of early studies of cross-species transmission; the viability of the source cysts was either unknown or assessed by eosin staining and, thus, failure of transmission could be due to feeding non-viable cysts, rather than an indicator of species specificity. Research prior to 1985 (ICAIR, 1984) suggest cross-species transmission can occur in some instances. In studies with *Giardia* cysts from humans, attempts were unsuccessful to infect hamsters, domestic rabbits, laboratory mice, deer mice, cattle, wapiti, mule deer, white-tailed deer, black bear, and domestic sheep. Success was reported in infecting laboratory rats, gerbils, guinea pigs, beavers, dogs, raccoons, bighorn sheep, and pronghorns with human-source *Giardia* cysts (ICAIR, 1984). It was also reported that two of the three human volunteers became cyst-positive after ingestion of *Giardia* cysts from a beaver, but when *Giardia* cysts were collected from beavers and fed to laboratory mice, rats, guinea pigs, and hamsters, none of these animals became infected (ICAIR, 1984). When cysts from the same beavers were given to four beagle puppies, all became cyst-positive, but these results cannot be interpreted because a control animal was also found positive. *Giardia* cysts

from a naturally-infected mule deer failed to infect two beagle puppies. Studies using *Giardia* cysts from asymptomatic and symptomatic human donors, as well as axenically cultured trophozoites originally isolated from a human, concluded: infection with *G. lamblia* is not restricted to humans; the trophozoite stage is also infectious; and household pets, particularly dogs, should be considered as a possible source of infection for humans (ICAIR, 1984).

Erlandsen et al.(1988a) studied the question, can *Giardia* cysts isolated from humans cause infections in animals? They tested the ability of human-source *Giardia* to infect beavers and muskrats. These investigators first showed that their cysts, from symptomatic human donors, were viable. Inoculation of 5×10^5 *G. lamblia* cysts resulted in infection in 75% of beavers. In some experiments, fewer than 50 cysts were sufficient to infect the beaver. In contrast, muskrats could only be infected with human-source *Giardia* when the dose was equal to or greater than 1.2×10^5. As a result of these studies, the authors concluded that the beaver and muskrat must be considered possible intermediate reservoirs for *Giardia* that infect humans. However, at that time, they were not able to assign to these animals a major role in the epidemiology of waterborne giardiasis in humans.

A polymerase chain reaction (PCR)-based method for genotyping *G. duodenalis* isolates using a polymorphic region near the 5' end of the small subunit (SSU) ribosomal RNA gene was described by Hopkins et al. (1997). Analysis was performed using *Giardia* cysts purified directly from feces. Isolates were collected from humans and dogs living in isolated Aboriginal communities in Australia where *Giardia* infections are highly endemic. This is the first report of the genetic characterization of *Giardia* from dogs and humans living in the same locality. Comparison of the SSU-rRNA sequences from 13 human and 9 dog isolates revealed four different genetic groups. Groups 1 and 2 contained all of the human isolates, whereas groups 3 and 4 consisted entirely of *Giardia* recovered from dogs. These results suggest that zoonotic transmission of *Giardia* infections between humans and dogs does not occur frequently in these communities. The dog-associated SSU-rRNA sequences have not been reported before,

suggesting a possible new *G. duodenalis* subgroup. A genetic basis for the differences observed between the groups was supported by sequence analysis of nine in vitro cultured isolates that were placed into the same genetic groups established by enzyme electrophoresis.

3. Transmission Between Animals

Information from studies using *Giardia*- free mice (ICAIR, 1984) demonstrated that *G. simoni, G. muris* and *G. peromysci* were host-specific while *G. microti* and *G. mesocricetus* were not. Pathogen-free mice were also successfully infected with *Giardia* from hamsters, but *Giardia* cysts obtained from parakeet feces and stored for 1-3 days were unable to infect mice or canaries (ICAIR, 1984).

Cross transmission studies in which beavers and muskrats were fed *Giardia* cysts from muskrats, beavers and mice were conducted by Erlandsen et al.(1988b). Beavers did not become infected when inoculated with cysts of *G. ondatrae* (source: muskrats) or *G. muris* (source: mice). Five of eight (62%) muskrats became infected when administered *Giardia* cysts of beaver origin.

4. Summary of Cross-Species Transmission

Many early *Giardia* transfer studies were poorly controlled, but more recent carefully controlled studies indicate that cross-species transmission of *Giardia* can occur. Experimental human and animal infection studies offer increasing evidence that some lower animals, particularly fur-bearing water mammals, are capable of harboring *Giardia* that can also infect humans (Isaac-Renton, 1994). While *Giardia* that are indistinguishable from those that infect humans are widespread throughout the Animal Kingdom, current evidence remains insufficient regarding their ability to be transferred to humans.

Experimental infection studies suggest that rats, mice, dogs, cats, beaver, muskrat, gerbils, and mule deer are capable of harboring *Giardia* that may also infect humans. The role in nature of these animals as a source of human infection, however, remains controversial. Of all of these animals, existing evidence suggests that the beaver and the muskrat are the most likely candidate mammals to serve as a source or reservoir of giardiasis and possible cause of some outbreaks in humans. Both of these aquatic mammals can be infected with isolates of *Giardia* from humans. However, each has also been shown to harbor strains of *Giardia* that are phenotypically distinct from those found in humans. *G. mictoti*, a species distinct from that in humans, has been found in muskrat (van Keulen et al., 1998). It is possible that the beaver harbors two types of *Giardia*. One type may be highly adapted to this animal and is rarely if ever transmitted to humans. The other type may be one acquired by the beaver from human sources, which can multiply in the beaver and in turn br transmitted via water back to humans. The argument supporting the complicity of the beaver and muskrat in human giardiasis and minimizing the role of other animals is as follows: In North America, epidemic and endemic giardiasis is frequently transmitted by contaminated water. See Section G, Chapter III for a further discussion of these studies. To deposit sufficient numbers of cysts that can infect large numbers of humans in a short time arguably is best accomplished by *Giardia*-infected animals which, by nature, defecate in fresh water. While cyst-bearing feces of rats, mice, dogs, cats and deer may occasionally reach drinking water, these animals do not, as beavers and muskrats do, by nature defecate in water. Beavers have been implicated as a possible source of contamination in several waterborne outbreaks (Craun, 1990). Thus, while *Giardia* that are indistinguishable from those that infect humans are widespread throughout the Animal Kingdom, current evidence remains insufficient regarding their ability to be transferred to humans.

To conclusively determine whether human giardiasis can be acquired by zoonotic routes and whether the ultimate source was human or a lower animal will require carefully controlled feeding studies and more detailed investigation of waterborne outbreaks that includes: systematic collection of *Giardia* cysts (1) from infected humans, (2) from animals suspected of

transmission, and (3) from environmental samples, and their characterization by molecular approaches such as zymodeme or karyotype identification. Studies taking this approach are in progress in British Columbia, and have strengthened the evidence that suggests a role for beaver in the spread of giardiasis to humans (Isaac-Renton, 1994).

Buret et al. (1990) postulated that domestic ruminants may be a reservoir for human infection. A study of *Giardia* infection of ruminants found that cyst output and clinical signs resembled human disease and that the *Giardia* from infected ruminants was morphologically and antigenically similar to humans. *Giardia* trophozoites from sheep were successfully cultured in TYI-S-33 medium; cytosolic, cytoskeletal, and membrane fractions were found to exhibit protein profiles similar to human isolates. Immunoblotting indicated that sera from infected sheep recognized human *Giardia*, and sera from human patients with giardiasis recognized *Giardia* from sheep. In both cases, recognition involved antigenic proteins of similar molecular weight. A pilot study of experimentally infected eastern barred bandicoots (*Perameles gunnii*) in Tasmania suggested their susceptibility to infection with *Giardia* from a human source (Bettiol et al., 1997).

E. Species Concepts in the Genus *Giardia*

In the past, there was no general agreement regarding the characteristics which define species in the genus *Giardia*. Characteristics used previously include host specificity, morphology, and variations in the shape of the median bodies (ICAIR, 1984). While *Giardia* size and shape may vary somewhat with the organisms collected from the respective host species, *Giardia* isolated from different host species may also be morphologically indistinguishable, and additional characteristics should be included in determining the speciation of *Giardia* (ICAIR, 1984). Based on work with *G. ardeae*, Erlandsen et al (1990b) stated that median body structure alone should no longer be considered adequate for classification at the species level. Their

axenic culture work was used to derive trophozoites, and the species description was based on a variety of morphological criteria and on chromosomal migration patterns.

1. Filice's Concept

In 1952, Filice concluded that the use of differences in body dimensions of *Giardia* and host specificity were untrustworthy criteria for distinguishing between species and suggested that *Giardia* morphological groups based primarily on structural differences. It was recognized, however, that physiologically distinct species may exist among those that appear similar morphologically. Filice proposed recognizing three *Giardia* groups:

(1) *G. duodenalis*, with a single or double median body which somewhat resembles the claw of a claw hammer. These organisms have been isolated from humans, other mammals (including rodents), birds, and reptiles.

(2) *G. muris*, with two small rounded median bodies in the center of the organism. Rodents, birds, and reptiles have been shown to be hosts for this morphological type of *Giardia.*

(3) *G. agilis*, with long, teardrop-shaped median bodies. While the adhesive disk of the other two proposed species is on the order of half the trophozoite body length, the *G. agilis* adhesive disk is only approximately one-fifth the body length of these organisms. Organisms of the *G. agilis* -type have only been described from amphibian hosts.

Filice also suggested assigning a non-taxonomic status, such as race, to allow incorporation of the *Giardia* that were considered to be distinct species on the basis of size or host specificity alone.

2. Grant and Woo's Concept

Grant and Woo (1978a, 1978b) and Erlandsen et al. (1990b) questioned the concept of *Giardia* speciation based only on median body structure. Grant and Woo felt that species of

Giardia should be defined using a combination of morphological, morphometric, and host-specificity criteria. They were able to distinguish five species of *Giardia* within small mammals in Ontario, Canada: *G. muris, G. mesocricetus, G. simoni, G. microti,* and *G. peromysci.* The five species were divided into two types (ICAIR, 1984). In type I, trophozoites have elongated nuclei in the posterior region of the sucking disk, the sucking disk occupies a large portion of the body, basal bodies are anterior to the nuclei, and the median bodies are round or oval and are located near the center of the body. Type I includes *G. muris* and *G. mesocricetus.* In type II, trophozoites are reported to be longer than wide, the sucking disk is in the anterior half of the body, the nuclei are in the central region of the disk and the median bodies are comma- or claw hammer-shaped. Type II trophozoites include *G. simoni, G. microti,* and *G. peromysci.* Cross-transmission studies using *Giardia*-free mice and rats were used to examine the host specificity of the two types of *Giardia* that were discerned morphologically. *G. simoni, G. muris,* and *G. peromysci* were host specific, but *G. microti* and *G. mesocricetus* were not. Grant and Woo were not able to distinguish any further characterization of these species within each type based on morphological observations but subsequently found statistically significant differences among the major dimensions of some trophozoites within and between types I and II. They also described a variety of problems dealing with *Giardia* speciation based on morphometrics including differences in size, general morphology, density of cytoplasmic staining, and relative proportions of cytoplasmic organelles of *Giardia* trophozoites under the influences of prefixation drying times.

3. Other Concepts for the Speciation of *Giardia*

With the advent of new biochemical, molecular, and genetic techniques, there has been considerable activity in characterizing *Giardia* speciation. Meloni et al. (1995) described the isoenzyme electrophoresis analysis studies (Baveja et al., 1986; Andrews et al., 1989; Homan et al., 1992), and recombinant DNA probe characterizations (Homan et al., 1992; van Keulen et al., 1992, 1993) which have been conducted with *Giardia* to attempt to characterize the speciation.

Based on the host-specificity of *Giardia*, the genus was characterized in 1926 as having over 40 species (ICAIR, 1984). In 1952, Filice proposed 3 groups based on its morphology. However, these present taxonomic groupings do not reflect the genetic and phenotypic heterogeneity within the species *G. lamblia*, and afford little information on which to estimate host specificity, infectivity, or virulence.

In general, organisms which are primarily clonal and reproduce by asexual reproduction are characterized by distinctive population structures, showing excess heterozygosity, association between independent genes (linkage disequilibrium), and a greater proportion of their genetic variation being distributed between as opposed to within, their populations (Meloni et al., 1995). Moreover, these types of clonal organisms (e.g., those with asexual reproduction) present problems in taxonomic characterization, because some of the typical biological species concepts do not apply to them. These factors contribute to the past difficulties in clarifying the correct species designation for the grouping(s) of interest from the perspective of human giardiasis.

Meloni et al.(1995) utilized enzyme electrophoresis techniques on 97 isolates of *G. duodenalis* collected from humans, cats, cattle, sheep, dogs, goat, beaver, and rats in Australia. The intent was to characterize the mode of reproduction, population structure, taxonomy, and zoonotic potential. From these enzyme electrophoresis studies, it was possible to identify 47 groupings of enzyme patterns (called zymodemes) based on their cluster patterns with each other. These zymodemes imply that the parasitic organisms have similar genetic structures, but the clonal lineages imply they are evolutionarily independent, and mean that the actual mode of reproduction cannot be inferred with confidence (and may not be strictly asexual). The information presented by Meloni et al.(1995) does not provide information on which to support the species concept of either Filice or Grant and Woo; rather, the evidence shows the complexity of attempting a specifically-clarified approach to presenting a species designation. Thus, at present, there does not exist a completely satisfying designation of the actual number of species

with the genus *Giardia*, and further research is needed in the areas of molecular biochemistry and genetic biology.

F. Summary

All of the organisms in the genus *Giardia* are binucleate, flagellated protozoan parasites which cause infection by attaching to the wall of the small intestine in the upper gastrointestinal tract of humans and other vertebrates. The parasites exist in trophozoite and cyst forms.

Giardia have been reported in a variety of mammals and in lower vertebrates. While numerous species of *Giardia* have been described, there is no general agreement on those criteria which define species in this genus. Criteria used to date include: host specificity; body size and shape, and the morphology of a microtubular organelle, the median body; and biochemical, molecular, and genetic techniques, such as the PCR for DNA-based detection and identification. The median body is an organelle that appears to be unique to *Giardia* trophozoites.

In the *Giardia* life cycle, the trophozoites divide by binary fission, attach to the brush border of the small intestinal epithelium, detach for unknown reasons, then become rounded and elaborate a cyst wall. The environmentaly-resistant cyst is excreted in the feces, and the transmission to a new host is accomplished by ingestion of viable cysts. The excystation process is initiated by conditions in the stomach, and is only completed once the excysting trophozoites pass into the less acidic conditions of the small intestine, where the trophozoites promptly attach to small intestinal epithelium.

Excystation is induced by exposure to low pH (as exists in the stomach), and has been induced in vitro. Encystment is initiated by exposure of trophozoites to bile (exact components unknown) in the upper bowel and continues as the excysting trophozoites pass to the lower small intestine, where the trophozoite rounds up and secretes cyst wall components which move in

vesicles to the cell wall to begin the process of cyst wall formation. This cyst wall protects the cysts when they pass out of the host with the feces. At that time, the cyst moves through the environment, primarily aquatic, and can possibly be transmitted to another vertebrate host.

Although many early studies were poorly controlled, more recent studies suggest that cross-species transmission of *Giardia* can occur. Experimental human and animal infection studies and information from waterborne outbreak investigations offer increasing evidence that beaver, muskrat, rats, mice, dogs, cats, gerbils and mule deer can be infected experimentally and may harbor human *Giardia;* in addition, humans appear to have been infected by *G. lamblia* cysts isolated from beaver and mule deer. Furthermore, it seems that these experimental infections may be established by direct-feeding with doses of either cysts or cultured trophozoites. Mice and rats, experimentally infected with *G. lamblia*, appear to produce a relatively low number of cysts. The necessity for meticulous attention to the use of *Giardia* -free animals is discussed. To determine the *Giardia*-free status in experimental animals, reliance on stool examination alone is not sufficient, because necropsy analyses have shown that trophozoites may be present in the intestinal tract in a higher percentage of the population than is revealed by relying solely on fecal analyses.

Of all of the animal species suspected of being a significant zoonotic source of human giardiasis, the evidence presently available suggests that the beaver and muskrat are the most likely candidates. The role of these animals as a source of human infection, however, remains controversial. Both aquatic mammals can be infected with isolates of *Giardia* from humans, but each has also been shown to harbor strains of *Giardia* that are phenotypically distinct from those found in humans. It is possible that the beaver harbors two types of *Giardia*. One type may be highly adapted to this animal and is rarely if ever transmitted to humans. The other type may be one acquired by the beaver from human sources, which can multiply in the beaver and in turn br transmitted via water back to humans.

III. OCCURRENCE

G. Worldwide Distribution

1. Distribution in Animal Populations

Organisms in the genus *Giardia* have been reported as intestinal inhabitants within a variety of mammals, birds, reptiles, amphibians, and fishes (ICAIR, 1984). Thus, *Giardia* should be considered among the most widely occurring of the intestinal protozoan parasites. Early workers assumed *Giardia* identified in animals was host specific. *Giardia* from some animals exhibit an apparent high degree of host specificity; other isolates may infect more than one host (Grant and Woo, 1978b; Davies and Hibler, 1979; Erlandsen et al., 1988a,b; Ey et al., 1997).

Giardia is a common protozoan parasite of farm animals and occurs with greater prevalence in young animals. Buret et al. (1990) found 18% of sheep and 10% of cattle infected with *Giardia*; a higher prevalence was found in lambs (36%) and calves (28%). Olson et al. (1997a) reported the following prevalence in farm livestock in Canada: cattle (29%), sheep (38%), pigs (9%), and horses (20%). A high prevalence of *Giardia* infection has been found in dogs (77%); a lower prevalence (3-11%) has been found in cats (Bemrick 1961; Kirkpatrick, 1986). A high prevalence of *Giardia* (>90%) has been reported for wading birds and muskrats (Erlandsen, 1994; Erlandsen et al., 1990b,c). *Giardia* have also been found in beaver (7-16%), voles, mice, shrews, native marsupials, ringed seals, and llamas. See Chapter IV, Section C for further information on the prevalence of *Giardia* in animals.

Surveys of *Giardia* in animal populations have often relied on detecting cysts in fecal samples. In a survey of beaver and muskrat populations from the northeastern United States and Minnesota, Erlandsen et al.(1990c) compared the analysis of fecal samples with the detection of internal trophozoites at necropsy. Beaver infection with *Giardia* was 9.2% (n = 662) by analysis

of cysts in fecal samples from kill-trapped samples and 13.7% (n = 302) by examination for intestinal trophozoites in live-trapped animals. For muskrat, the differences were even greater, with the prevalence of *Giardia* 36.6% (n = 790) by detection in fecal samples from kill-trapped animals and 95.9% (n = 219) by examination of the intestinal contents. These data suggest that surveys based on fecal analyses may under-report the actual numbers of infected animals.

2. Distribution in Human Populations

Giardiasis is the most commonly reported intestinal protozoan infection worldwide. The World Health Organization estimates 200 million people are infected each year (Swarbrick et al. 1997). Human infections with *Giardia* have been reported in all of the major climatic regions from the tropics to the arctic (ICAIR, 1984). All age groups are affected, but children are more frequently infected than adults (Benenson, 1995). An analysis of analyzed randomly collected stool specimens in two counties of Washington State found that 7.1% of 515 healthy 1- to 3-year-old children were positive for *Giardia* cysts (ICAIR, 1984). In the United States, United Kingdom, and Mexico, endemic infection most commonly occurs during July to October and among children under five years of age and adults aged 25-39 years of age (Benenson, 1995). The prevalence of stool positivity may range from 1-40%, depending on the geographic area and age group surveyed; prevalence is higher in areas with poor sanitation and institutions with children not toilet trained (Benenson, 1995). The prevalence of infection can be as high as 35% among children attending child care centers with attack rates in outbreaks of 50% or more (Adam, 1991; Hall, 1994; Ortega and Adam, 1997; Steketee et al., 1989). Prevalence rates vary from 2-5% in developed countries; in developing countries, prevalence can be as high as 20-40% (Farthing, 1996). All of a birth cohort of 45 Guatemalan children had giardiasis before age four (Farthing, 1996) and 40% of Peruvian children were infected by six months of age (Ortega and Adam, 1997; Miotti et al., 1986). See Chapter III, Section F for further discussion.

The Centers for Disease Control and Prevention (CDC) do not require notification or

reporting of cases of giardiasis. Localities, states, and U.S. territories conduct their own disease surveillance and voluntarily report cases of giardiasis to the CDC. Forty-three states, the District of Columbia, and three U.S. territories have mandatory reporting requirements for giardiasis (Chorba et al., 1989). Giardiasis has been reported in the District of Columbia and the following 40 states and 4 territories: Alaska, Arizona, Arkansas, California, Colorado, Connecticut, Delaware, District of Columbia, Florida, Guam, Hawaii, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maine, Maryland, Minnesota, Mississippi, Missouri, Montana, New Hampshire, New Jersey, New Mexico, New York, Ohio, Oklahoma, Oregon, Pacific Trust Territory, Pennsylvania, Puerto Rico, South Carolina, South Dakota, Tennessee, Texas, Utah, Vermont, Virgin Islands, Virginia, Washington, West Virginia, Wisconsin (ICAIR, 1984).

In a non-random survey of 332,312 stool specimens from patients submitted for parasitological evaluation in 1978 by 53 state and territorial public health laboratories (ICAIR, 1984), the CDC found that *Giardia* was the most commonly identified parasite in the United States. The percentage of *Giardia*-positive stools ranged from 1.1% of specimens in Virginia to greater than 8% of specimens in Arizona, California, and Washington (ICAIR, 1984). *Giardia* was also the most frequently identified parasite in laboratory surveys conducted in 1976 and 1977 in the United States (Kappus et al., 1994) and in 1979 in Canada (Gyorkos, 1983). In 1984, 12.5% of 1,710 public health clinic patients seen in Oregon were found positive for *Giardia* (Skeels et al., 1986). National surveys were also conducted in 1987 and 1991 with 49 states participating (Kappus et al., 1994). *G. lamblia* continued to be the most frequently identified parasite. It was found in 7.2% of 216,675 specimens examined in 1987 and 5.6% of 178,786 specimens examined in 1991, an increase from the 3.8% to 4.0% average found in the 1976, 1977, and 1978 surveys. Forty states reported an increased identification in specimens submitted to the laboratories. Seasonally, *Giardia* identifications increased in the summer and fall, especially in the Midwest. States reporting high percentages of *Giardia* identification for both 1987 and 1991 were located in the Midwest or Northwest. In 39 states and eight of the nine regions, *Giardia* was the most frequently identified parasite every month of the survey periods.

An analysis of hospital discharge data from the National Center for Health Statistics (Lengerich et al., 1994) estimated that 4,600 persons were hospitalized with giardiasis annually in the United States from 1979 to 1988 -- an incidence of 2.0 hospitalizations per 100,000 persons per year. The estimated hospitalization rates were highest for children less than 5-years-old (4.6 per 100,000 per year) and women of child-bearing age, 25- to 34-years-old (3.5 per 100,000 per year). Although children younger than 5 years of age also had a high rate of hospitalization in Scotland, women of child-bearing age were no more likely to be hospitalized with giardiasis than men of the same age group (Robertson, 1996). Among residents of Michigan from 1983 to 1987, the average incidence of hospitalization was 1.4 per 100,000 persons per year (Lengerich et al., 1994). Hospital admissions for giardiasis account for only one-tenth of all cases of giardiasis reported by physicians and only a fraction of symptomatic and asymptomatic cases are seen by physicians (Lengerich et al., 1994).

B. Occurrence in Water

Giardia cysts are ubiquitous in surface waters of all qualities. Because *Giardia* infections are widespread in human and animal populations, contamination of the environment is inevitable and cysts have been detected in even the most pristine of surface waters. The limitations of the detection methodology with respect to efficiency of recovery and viability or infectivity of the detected organisms should be borne in mind when evaluating the significance of occurrence data. See Chapter VII, Section A for a further discussion.

1. Wastewaters

Wastewaters that are discharged into rivers and streams are sources of *Giardia* in surface waters. Sykora et al. (1991) studied the occurrence of *Giardia* in the wastewaters and sludges of 11 cities across the continental United States. In examining monthly samples from each site,

they found that all of the raw sewage samples were positive for cysts at levels ranging from 4 cysts/L to 14,000 cysts/L. The geometric mean level of cysts at each site ranged from 642/L at a Pennsylvania treatment plant to 3,375/L at a California plant. A seasonal distribution was noted with cyst levels reported highest during late summer through early winter. While all of the raw sewage samples were positive, about 48% of the secondary effluents contained cysts at levels ranging from 1 to 44/L. About 80% of the sludges were positive at levels ranging from 70 to 30,000 cysts/L. It should be recognized that the cyst counts in this study can be considered minimal because the analyses used the iodine staining method which has been shown to produce significantly lower cyst counts than an immunofluorescence assay (IFA) applied to environmental samples (LeChevallier et al., 1990).

Investigators in Scotland examined eight raw sewage samples collected from November, 1990 to January, 1991 (Smith et al., 1994). Cyst levels ranged from 38 to 44 cysts/L, but the method used to examine the samples was not specified. Seven effluent samples were also examined with cyst levels ranging from 0.8 to 5.9/L. A subsequent investigation in Scotland examined raw sewage samples collected bimonthly for one year from six sewage treatment plants (Robertson et al.,1995). All of the samples were positive for cysts at levels ranging from 102 to 43,907/L.

Roach et al. (1993) examined raw and treated sewage samples collected from five sites in the Yukon. Forty four samples of raw sewage were examined, and all were positive for *Giardia* cysts at levels ranging from 26 to 3,022 cysts/L. Five samples of treated sewage from two locations were positive at cyst levels ranging from 2 to 3,511 cysts/L. Daily monitoring of raw sewage at one site suggested an increasing level of giardiasis in the community during the summer months that was possibly related to episodic occurrence of cysts in the water supply in the spring and early summer (Roach et al., 1993).

Enriquez et al. (1995) used IFA to examine 130 wastewater samples from two activated sludge treatment plants and a water reclamation plant for *Giardia* over a three year period. The geometric mean level of cysts in secondary effluents from each of two activated sludge treatment plants was 8.3 and 6.6 cysts/40 L. The geometric mean cyst level in the tertiary effluent from the water reclamation plant was 3 cysts/40 L. No seasonal variation in cyst levels was observed.

Grimason et al. (1996) reported finding *Giardia* cysts in 37% of raw sewage samples collected at a plant in Kenya and in 100% of similar samples collected at a plant in southern France. These treatment plants processed municipal wastewater using stabilization pond systems. Cyst levels ranged from 1,000 to 25,000 cysts/L in the Kenyan sewage and from 230 to 25,000 cysts/L in the French sewage. Cysts were detected in all final pond effluents at the Kenyan plant and in 44% of those sampled at the French plant. Calculations indicated that pond retention periods of 25 to 40 days would remove more than 99% of the cysts but were not sufficient to assure cyst-free effluents. Wiandt et al. (1995) evaluated seasonal differences in cyst levels in the final effluent at the same French plant. They were unable to detect cysts in the final effluent in samples collected during the spring and summer but did find cysts at levels of 0.1 to 2.5/L during the wintertime.

In evaluating IFA and polymerase chain reaction (PCR) methods for detecting cysts, Mayer and Palmer (1996) examined 11 samples of raw sewage, 11 samples of primary effluent and 10 samples of secondary effluent at a large metropolitan treatment plant in California. They reported levels of 1.3×10^4, 2.6×10^3 and 1.1×10^1 cysts/L in the primary influent, primary effluent and secondary effluent samples, respectively.

Hirata and Hashimoto (1997) reported a survey on the occurrence of *Giardia* and *Cryptosporidium* in nine activated sludge sewage treatment plants located in the Kanto area of Japan. Grab samples were centrifuged, the pellets were purified by flotation and the cysts were detected and enumerated using an immunofluorescence assay. Viability was not determined.

The limit of detection of their methods was about 16 cysts/L for raw sewage and primary effluents and 2 cysts/L for secondary and final effluents. They found 95% (112/118) of the samples positive for cysts: 100% (29/29) of the raw sewage samples (geometric mean = 1,500/L; range = 130 to 7,900/L); 100% (37/37) of the primary effluent samples (geometric mean = 1,100/L; range = 150 to 6,600/L); 86% (30/35) of the secondary effluent samples (geometric mean = 18; range = 2 to 310), and 82% (27/33) of the final effluent samples (geometric mean = 14/L; range, 4 to 130/L). The average removal of cysts by the activated sludge treatment process was 1.8 $\log_{10}$. The level of *Giardia* cysts was significantly correlated with coliforms, *Escherichia coli, Clostridium perfringens*, and turbidity. Turbidity was also correlated with removal of cysts by the treatment process.

Examination of *Giardia* in raw sewage has been suggested as an alternative method of assessing the prevalence of *Giardia* infection and detecting possible outbreaks in communities, but only one research study has examined the relationship between cysts in sewage and illness in the community. A correlation was found between raw sewage cyst levels and reported cases of giardiasis in 11 communities in the United States (Jakubowski et al., 1991).

2. Surface Waters

Numerous studies have been directed at determining levels of *Giardia* in drinking waters and the surface waters that serve as the source waters for drinking waters to help assess the risks of waterborne *Giardia* infection in populations using these waters. In the United States, studies have been conducted to collect water samples over large geographical areas and within individual drainage basins; studies have also been conducted in Canada, the Virgin Islands, Europe, and Asia (Table III-1). Authors of original studies reported various units, but in presenting the results of these studies, data have been converted and presented here for consistency as "cysts/100 L."

The largest single data base of water samples examined for *Giardia* was developed and compiled by Hibler (1988). He analyzed 4,423 water samples from 301 municipal sites in 28 states between 1979 and 1986 using a zinc sulfate flotation/iodine staining method to detect cysts (APHA-AWWA-WPCF, 1981). He reported his results as percentages of various types of samples found positive for cysts and did not include quantitative data on cyst levels. He found 34% of the municipal sites (102/301) were sometimes positive for cysts, with 26% (512/1,968) of raw water samples and 11% (267/2,732) of finished water samples demonstrating cysts. In order of decreasing prevalence, the positivity rates for source water types were 28% (346/1,218) for creeks; 26% (212/828) for rivers, 10% (193/1,983) for lakes, 19% (16/84) for springs, and 3% (2/63) for wells.

Rose et al. (1988a) conducted a biweekly survey of a watershed in the western United States for a one-year period to sample for the presence of *Giardia* cysts and *Cryptosporidium* oocysts. *Giardia* cysts were detected in 31% (12/39) of samples collected from a lake receiving sewage effluents and a river beginning at the lake and running through an area where there were a number of cattle pastures. Higher *Giardia* cyst levels were found in the river downstream from the cattle pastures: a mean level of 22 cysts/100 L, and a range from 0 to 625 cysts/100 L. The geometric mean *Giardia* cyst levels detected in the lake was 8 cysts/100 L with a range of 0 to 222 cysts/100 L. A seasonal variation was found. Geometric means for the summer, fall, winter, and spring were 35/100 L, 31/100 L, 0.7/100 L, and 0.1/100 L, respectively. *Giardia* cyst levels were significantly correlated with *Cryptosporidium* oocyst levels, but no significant correlations were observed between cyst levels and either total or fecal coliforms or turbidity.

Ongerth (1989a) conducted a study to assess *Giardia* cyst levels in three pristine watersheds in Washington state. A membrane filter sampling method was utilized with 40 L samples collected in the field and returned to the laboratory. Cyst recovery efficiencies were measured (average = 21.8% ± 6%; range = 5-44%). Reported cyst levels were calculated based on the percentage recovery measured in the positive controls, as well as the more routinely

applied calculation using the percentage of the microscope slide counted. *Giardia* cysts were found in 94 (43%) of the 222 samples. Based on the recovery efficiencies and sample volume, the cyst levels ranged from 10 to 520 cysts/100 L. The median levels were extrapolated for the three rivers, with the Green, Cedar, and Tolt Rivers being 6/100 L, 4/100 L, and 0.3/100 L, respectively; distributions of cyst values were reported to be lognormal, but the cyst level (based on mean values) and its variability (based on the slopes of the distributions) differed among the rivers. Samples were also collected in tributaries to each of the three rivers, and the levels were generally lower than found in the main-stem sites for each river. In addition, samples in each river were collected above and below impoundments, and levels were generally higher below the impoundments than above, although the differences were not statistically significant. No statistically significant seasonal variations were observed over the nine-month sampling period; levels varied in each river by less than a factor of 2. Ongerth (1989a) concluded that *Giardia* cysts, even though present at low levels, appear to be continuously present in these relatively pristine rivers.

Rose et al. (1991a) detected *Giardia* cysts in 16% of 257 water samples from 17 states at an average level of 3 cysts/100 L. The arithmetic average of *Giardia* cysts ranged from <1 to 140 cysts/100 L, depending on the nature of the waters being sampled. The geometric mean was 4 cysts/100 L for all rivers. 11 cysts/100 L (maximum = 625/100 L) for polluted rivers, and 0.35 cysts/100 L (maximum = 12/100 L) for pristine rivers. For lakes, the geometric mean was similar to rivers, 3 cysts/100 L. The geometric mean was 6.5 cysts/100 L (maximum = 156/100 L) for polluted lakes and 0.5 cysts/100 L (maximum = 7/100 L) for pristine lakes. *Giardia* cysts were not detected in any of the drinking water samples. Levels of *Giardia* cysts were not found to be correlated with turbidity, total coliform or fecal coliform water quality indicators. Samples collected in the fall were more likely to be positive than samples collected during other seasons and cyst levels tended to be higher in summer/fall samples than in winter/spring samples.

LeChevallier et al. (1991a) sampled the source waters at 66 surface water treatment plants in 14 states and one Canadian Province. *Giardia* cysts were found in 81.2% (69/85) of raw water samples with levels ranging from 4 to 6,600 cysts/100 L; the geometric mean of positive samples was 277 cysts/100 L. About 13% of the 618 *Giardia* cysts observed in raw water samples demonstrated morphological characteristics suggesting that these cysts may be viable. Higher cyst densities were associated with watersheds receiving decreasing protection. The authors noted that sources receiving industrial (urban) pollution contained 10 times more *Giardia* cysts than protected watersheds. Unlike the Rose et al. (1988a, 1991a) results, LeChevallier et al. (1991a) found a significant relationship between *Giardia* cyst densities and total coliforms or fecal coliforms, postulating that this difference might have been due to the types of water samples analyzed in each study. Rose et al. (1988a, 1991a) examined relatively pristine waters while LeChevallier et al. (1991a) examined a variety of source waters, including those with high levels of pollution as demonstrated by bacterial indicator counts. LeChevallier et al. (1991a) concluded that as levels of pollution increase as indicated by higher bacterial indicator counts and turbidities there is an increasing probability of the presence of *Giardia* cysts at higher densities.

Table III-1. Reported Data for *Giardia* in Surface Waters

Sample	No. of Samples (n)	Positive Samples (%)	Detection Limit (cysts/100 L)	Recovery Efficiency (%)	Mean Level of Cysts[a] (cysts/100 L)	Range of Cyst Levels (cysts/100 L)	Viability (%)	Reference
River (downstream from cattle pastures)	19	21			22	0-625		Rose et al., 1988a
Lake (received sewage effluents; upstream of cattle pastures)	20	40			8	0-222		
Pristine rivers in WA: Green Cedar Tolt	222	42		22	 6 (median) 4(median) 0.3(median)	10-520		Ongerth, 1989a
17 states in western US: Polluted Rivers Pristine Rivers Polluted Lakes Pristine Lakes	257 38 59 24 34	16 26 7 33 12			3 11 0.35 6.5 0.5	<1-625 <1-625 <1-12 <1-156 <1-0.5		Rose et al., 1991a
66 water treatment plants in 14 states and 1 Canadian province: Raw surface waters Filtered drinking waters	 85 83	 81 17			 277 4.45	 4-6,600 0.29-64	 13[a] 13[a]	LeChevallier et al., 1991a LeChevallier et al, 1991b

Sample	No. of Samples (n)	Positive Samples (%)	Detection Limit (cysts/100 L)	Recovery Efficiency (%)	Mean Level of Cysts[a] (cysts/100 L)	Range of Cyst Levels (cysts/100 L)	Viability (%)	Reference		
Continuation of LeChevallier et al. 1991a										
Raw surface waters:										
1988-1993	347	54		42(<1 NTU)	200	2-4,380	15[b]	LeChevallier and Norton, 1995		
1991-1993	262	45		50(150 NTU)						
Three water treatment plants in Quebec, Canada										
Raw water	17	94			7-1,376	<2-2,800		Payment and Franco, 1993		
Settled water	74	66			0.14-0.4	<1-15				
Filtered water	34	3			0.01-<.2	<0.1-0.1				
Finished water	31	3			0.2	<0.1-0.7				
Yukon Surface waters								Roach et al., 1993		
Pristine	22	32								
Drinking water intake	42	17				<1-1.4				
Ottawa, Ontario rivers	41	78			8	<1-52		Chauret et al.,1995		
Lakes/rivers	147	23			210	40-630		Norton et al., 1995		
WI waters: Streams	210	31(48 in winter; 25-29 other) 5(11 in spring)			38(median)	0-2,610		Archer et al., 1995		
Lakes	179				1.2(2.6 in spring, medians)	0-125				

Sample	No. of Samples (n)	Positive Samples (%)	Detection Limit (cysts/100 L)	Recovery Efficiency (%)	Mean Level of Cysts[a] (cysts/100 L)	Range of Cyst Levels (cysts/100 L)	Viability (%)	Reference		
86 sites in British Columbia, Canada:							34[c]	Isaac-Renton et al., 1996		
Raw water	153	64			2.9					
Finished water	91	59			2.1					
2 community supplies:										
BMID Raw							11[c]			
	70	100			229	7-2,215				
Reservoir settled	75	99			95	12-626				
Chlorinated	77	77			31	0.3-371				
Tap	7	71			?	1.5-18.5				
VID										
Raw	53	100			30	8-114				
Chlorinated	53	98			14	2-73				
Treatment plant in Germany:					(medians)			Karanis et al., 1996a		
Raw water	12	83			24.5	2-103				
Backwash water	50	84			22.3-55	3-374				
PA rivers:								States et al., 1997		
Allegheny R.	24	63			34	12-421				
Youghiogeny R.	24	54			118	44-526				
Dairy farm stream	22	55			82	13-1,527				
Settled water	23	8			29	12-70				
Filtered water	24	0								
Backwash water	22	8			59	15-237				

Sample	No. of Samples (n)	Positive Samples (%)	Detection Limit (cysts/100 L)	Recovery Efficiency (%)	Mean Level of Cysts[a] (cysts/100 L)	Range of Cyst Levels (cysts/100 L)	Viability (%)	Reference
NJ reservoirs:				39			20[b]	LeChevallier et al., 1997
Inlet	60	13	2.4		1.9	0.7-2.4		
Outlet	60	15	6.2		6.1	1.2-107		
PA filter plants:								Consonery et al., 1997
Raw water intakes	148	23			0.23	0.04-5.7		
Finished water	148	0						
2 CA watersheds								Stewart et al., 1997
Sampling method:								
First flush (5 L, storm)	20	60				25-16,666		
Filter (100 L ,non-storm)	87	29				2-119		
Grab (4 L, storm, non-first flush)	21	19				42-2,428		
NY reservoirs:								Okun et al., 1997
Catskill		36			1.2	9.3(max.)		
Delaware		29			0.7	8.2(max.)		
Malcolm Brooks		46			1.3	23.4(max.)		
Selangor, Malaysia, 2 plants:								Ahmad et al., 1997
Raw water	20	90				100-2,140		
Treated water	20	0						
6 surface water treatment plants in Germany:								Karanis et al., 1998
Raw water	105	64			88.2(?)	1,314 (max.)		
Treated water	150	20			2.9(?)	19.2 (max.)		
Drinking water	47	15			3.8(?)	16.8 (max.)		

[a] Geometric mean unless otherwise specified.
[b] By morphological criteria
[c] By mouse infectivity

LeChevallier et al. (1991b) also conducted analyses of treated water samples and reported that cysts were detected in 17% of the 83 finished drinking water samples collected from the same 66 surface water treatment plants. A viable type morphology was demonstrated by 13.3% of the 46 *Giardia* cysts that were examined from these samples. The authors explained that this does not mean the cysts were alive, but instead, that cysts which were distorted or shrunken were probably dead. The communities served by the treatment systems in which the cysts were found did not experience any apparent outbreaks of giardiasis, but the disease and waterborne outbreak surveillance activities were not described for these communities. The investigators indicated that 24% of the treatment plants examined would not meet the risk level specified in the Surface Water Treatment Rule (SWTR), and under cold water conditions (0.5°C) 46% of the plants would not meet the risk level (U.S. EPA, 1989a). See Chapter VI for a discussion of the recommended *Giardia* infection risk level.

In a subsequent study, LeChevallier and Norton (1995) updated their previous studies from 1991, and presented data from an extensive monitoring program of drinking water source waters. The levels of *Giardia* cysts were determined in a total of 347 surface water samples collected from 1988 through 1993 at 72 treatment plants in 15 states and 2 Canadian provinces. *Giardia* cysts were detected in 53.9% of the samples. This compares to 81.2% of samples positive in their previous study. The authors dismissed method differences as possible explanations for the decreased prevalence and suggested that occurrence levels may fluctuate due to unknown causes or that there may have been an actual decline in levels over the four year period examined. For the 1991 to 1993 sampling period, *Giardia* cysts were detected in 118 (45.0%) of 262 raw water samples. The geometric mean of positive samples was 200 cysts/100 L, ranging from 2 to 4,380 cysts/100 L. Based on microscopic examination, 14.6% (50/343) of the cysts had potentially viable morphology. *Giardia* cysts were detected in 4.6% (12/262) of filtered plant effluent samples at levels ranging from 0.98 to 9 cysts/100 L (mean = 2.6 cysts/100 L) during the 1991 to 1993 period. Because microscopic examination suggested that the majority

of the organisms were dead, the authors concluded that there was little reason to believe that any of the communities served by these plants was at risk for a waterborne outbreak of giardiasis.

Roach et al. (1993) analyzed for *Giardia* cysts in samples collected in Yukon surface waters in the Canadian North. Pristine streams were sampled, and cysts found in 32% (7/22) of the samples. A sample taken through the ice at Lake Laberge contained 7.5 cysts/100 L, and this was the highest level found in the surface waters included in the study. The drinking water intakes for two communities, Whitehorse and Dawson, were also sampled, and cysts were detected in 17% (7/42) of the Whitehorse samples, but in none of the ten Dawson samples. Six of the seven positive Whitehorse samples contained about 0.2 cysts/100 L, and one contained about 1.4/100 L.

Payment and Franco (1993) sampled raw, settled, filtered and finished water from three drinking water treatment plants in the Montreal, Canada, area. Two of the plants used the same river for a source and the third used another river. For the raw water samples, 94% (16/17) were positive at levels ranging up to 2,800 cysts/100 L. Settled water samples were positive 66% (49/74) of the time at cyst levels up to 15/100 L, but only one (3%) of 34 filtered water samples was positive at 0.1 cyst/100 L. Similarly, 3% of finished water samples (1/31) were positive for *Giardia* (0.7 cysts/100 L).

Chauret et al. (1995) examined raw water samples from two Ottawa, Canada watersheds characterized as "relatively pristine". They examined 41 raw water samples collected during the summer months at 15 sites along the Ottawa River (23 samples) and Rideau River (18 samples) watersheds. *Giardia* cysts were detected in 78% of samples at levels ranging from 1 to 52 cysts/100 L. An additional 12 raw water samples were collected at the intakes to two drinking water treatment plants located on the Ottawa River. They found 83% of these samples positive for cysts at levels up to 25/100 L, but none of an unspecified number of treated water samples were positive. No significant correlation was found between the presence of *Giardia* and any of

the microbial indicator examined (fecal and total coliforms, fecal streptococci, *Aeromonas* sp., *Pseudomonas aeruginosa, Clostridium perfringens*, algae and coliphages).

In another examination of pristine watersheds, Ongerth et al. (1995) collected duplicate samples from five locations on each of two rivers in the Olympic Mountains, Washington. They found greater numbers of *Giardia* cysts in water samples from the more heavily used watershed compared to the adjacent watershed that experienced lesser human use. The authors reported a relationship between the numbers of cysts in the water, the prevalence of cysts in selected animal species, and the extent of human use of the watershed area. The numbers of cysts found in the water samples ranged from 0.2/100 L to 3/100 L. Based on the water samples they analyzed, they calculated that a median cyst level of 1 cyst/20 L can be expected in relatively pristine waters.

In an investigation of Wisconsin waters, Archer et al. (1995) found *Giardia* at least once in all 18 streams examined and in 30.9% (65/210) of all stream samples. The highest level in a stream was 2,601 cysts/100 L from a site characterized as pristine. However, there was no relationship between land use types and presence or absence of *Giardia* on a State-wide basis. Although almost half of all samples collected in the winter were positive compared to about 25% of samples collected during other seasons, the authors indicated that seasonal differences were only weakly statistically significant (P=0.08). *Giardia* were not detected in any of 17 well water samples collected from six wells. Cysts were found in 5% (9/179) drinking water intake samples at levels ranging to 125 cysts/100 L (median, 1.2/100 L). None of 28 drinking water intake samples collected during the summer were positive for cysts.

Norton et al. (1995) examined 147 samples from 15 sites in New Jersey source waters for *Giardia*. These sites constituted 45% of the water supply for New Jersey. Sampling was conducted over a one-year period during 10 sampling events. The mean cyst level in the 23% of samples that were positive was 210/100 L; the range was 40 to 630/100 L.

Isaac-Renton et al.(1996) surveyed 86 sites throughout British Columbia, Canada for *Giardia* over a 12-month period. Although none of sites were downstream of large urban sewage discharges, they were not considered pristine because they were open to public access and many had agricultural activities in the vicinity. Of 153 raw water samples, 64% were positive for cysts at geometric mean levels of 2.9/100 L. These positive samples came from 69% of the sites. No seasonal variation was observed in raw water cyst levels. The 91 chlorinated drinking water samples examined were positive 59% of the time at a geometric mean level of 2.1 cysts/100 L. Thirty four percent (45/133) of cyst-positive samples inoculated into gerbils produced infection, and the frequency of infectivity was higher when raw water concentrates were inoculated as compared to treated water concentrates.

Isaac-Renton et al. (1996) also studied two community drinking water supplies in detail over a 2-year period. One community, the Black Mountain Irrigation District (BMID), was selected because it had previously been determined to have *Giardia* cysts more frequently and at higher levels than most other sites examined. The second community, the Vernon Irrigation District (VID), was selected because it frequently contained *Giardia* cysts but at lower levels than the BMID supply. For BMID, 100% (70/70) of raw water samples were positive at cyst levels ranging from 7 to 2,215/100 L; the geometric mean was 229/100 L. In samples collected after reservoir settling, 99% (74/75) were positive with levels ranging from 12 to 626/100 L; the geometric mean was 95/100 L. Chlorinated water samples were positive 77% (59/77) of the time with levels ranging from 0.3 to 371/100 L; the geometric mean was 31/100 L. Seventy one percent (5/7) of tap water samples were positive at levels from 1.5 to 18.5 cysts/100 L. A seasonal trend was noted with peak levels occurring in late autumn and early winter. Eleven percent (11/125) of cyst-positive concentrates were infective for gerbils. Infectivity of concentrates was less for settled water than for raw water and none of the chlorinated concentrates inoculated into gerbils produced infections. For the VID, 100% (53/53) of raw water samples were positive at 8 to 114 cysts/100 L; the geometric mean was 30/100 L. Chlorinated water samples produced 98% (53/54) positives containing 2 to 73 cysts/100 L; the

geometric mean was 14/100 L. No clear seasonal trend was observed in the VID samples. None of 66 samples positive for cysts and inoculated into gerbils produced infections. In both communities, cyst levels that spiked, i.e., rose to considerably higher values on a particular sampling date than was indicated by the trend, were observed. Ong et al. (1996) further evaluated sources of contamination of the watersheds in these two communities and found significantly higher levels of cysts in raw water from a creek downstream of a cattle ranch as compared to upstream samples in the BMID watershed. Differences were found in the percentage of cattle infected on both watersheds and the authors indicated the importance of good watershed management practices to providing best-quality drinking water supplies.

Wallis et al. (1996) analyzed 1,760 raw water, treated water, and raw sewage samples from 72 cities across Canada for *Giardia* cysts. Fifty-eight of the municipalities treated their drinking water by chlorination alone. The authors found *Giardia* cysts in 73% of the raw sewage samples, 21% of the raw water samples, and 18% of the treated water samples. Water samples from 74% (53/72) of the municipalities investigated were positive for cysts at least once. Detailed quantitative data were not supplied but the authors indicated that most of the water samples contained fewer than 2 cysts/100 L. The highest level of cysts encountered in a water sample was 230 cysts/100 L found in a community experiencing an outbreak of waterborne giardiasis. Sewage samples contained up to 88,000 cysts/L but the mode was under 1,000/L. Cysts infective for gerbils were detected in 2.2% (5/223) of raw water samples and in 7.6% (6/79) of treated water samples. *Giardia* cysts were recovered more frequently in late winter-early spring and fall and in higher levels than in other seasons, although the cysts were recovered in all seasons. By biotyping and karyotyping analyses, the cysts recovered were genetically similar to those recovered in other areas of the world. These authors reported that in Canada *Cryptosporidium* oocysts were less common than *Giardia* cysts. Based on monitoring data from waterborne outbreak investigations, they proposed an "action level" of three to five cysts/100 L.

States et al. (1997) conducted sampling in the vicinity of Pittsburgh, PA, in the Allegheny and Youghiogheny Rivers. Samples were collected monthly for two years. The occurrence of positive samples and the geometric mean values of detected *Giardia* cysts were, respectively, as follows: in the Allegheny River, 63% and 34 cysts/100 L; in the Youghiogheny River, 54% and 118 cysts/100 L. Sources of cysts in the Allegheny River were identified as combined sewer overflows (CSOs), a stream running through a dairy farm, and a sewage treatment plant. Samples were collected from CSOs during five storm events. All samples were positive and the cyst levels ranged from 3,750 to 114,000/100 L; the geometric mean was 28,681/100 L. In the stream running through the dairy farm, 55% (12/22) samples were positive at cyst levels of 13 to 1,527/100 L (geometric mean = 82/100 L). Effluents from the sewage treatment plant were positive 83% (20/24) of the time with densities of 102 to 4,614 cysts/100 L (geometric mean = 664 cysts/100 L). Filter backwash samples from the drinking water treatment plant were positive 8% (3/22) of the time at levels of 15 to 237 cysts/100 L (geometric mean = 59/100 L).

LeChevallier et al. (1997) studied the potential for contamination of six open finished water reservoirs in New Jersey. Ten samples of water from the influent and 10 samples of the effluent were collected over a 12 month period at each of the six reservoirs. Five of the reservoirs received finished water from conventionally filtered water plants, and the sixth received water from a high quality unfiltered surface water system. All of the reservoirs had fences but holes in the fences and evidence of human activity was evident at some of the sites. Thirteen percent (8/60) of the influent samples were positive for cysts at geometric mean levels of 1.9/100 L (median = 1.6). Of the effluent samples 15% (9/60) were positive at a geometric mean of 6.1/100 L (median = 6.0). The authors indicated that most cysts were probably non-viable based on morphological considerations. Twenty percent (9/45) of the cysts had potentially viable morphology; the remainder were empty shells and were probably non-viable. The differences in *Giardia* cyst levels between the influent and effluent samples were not statistically significant. The authors assumed that the differences in levels were most likely due to indigenous animal activity in the vicinity of the reservoirs.

Stewart et al. (1997) studied three sample collection methods for storm events in two California watersheds. Filter samples (100 L), first-flush samples (5 L) and grab samples (4 L) were collected. The first-flush samples had the highest positivity rate, 60% (12/20) and also had the highest cyst levels ranging from 25 to 16,666/100 L). Filter samples had an intermediate rate, 29% (25/87) with the lowest cyst levels (2 to 119/100 L). Only 19% (4/21) of the grab samples had cysts, but the cysts (42-2,428) were at intermediate levels. There was a seasonal effect with 45% of 31 filter samples positive during October to January sampling, while only 20% of 56 samples were positive during the February to July collection period. The authors concluded that peak levels of *Giardia* occur following storm events, especially the first storm of the season. An unattended first-flush sampling device was effective and minimized logistical sampling problems.

Okun et al. (1997) summarized the results of the New York City Department of Environmental Protection monitoring for *Giardia* between June, 1992 and January, 1995 of three of the City's drinking water reservoirs (Catskill, Delaware and Malcolm Brooks). The City's supply is not filtered and the source water was sampled prior to chlorination. Positivity rates of 36%, 29% and 46% were found for the Catskill, Delaware and Malcolm Brooks reservoirs , respectively. Cysts were found at mean levels of 1.2/100 L (maximum = 9.3/100 L), 0.7/100 L (maximum = 8.2/100 L) and 1.3/100 L (maximum = 23.4/100 L) at the reservoirs, respectively.

Raw and finished water samples from surface water treatment plants in Pennsylvania were examined intensively by Consonery et al. (1997) for *Giardia* between 1994 and 1996 as part of Pennsylvania's Filter Plant Performance Evaluation (FPPE). Cyst levels ranging from 0.4 to 5.7 cysts/100 L (mean = 0.23/100 L) were found in 23% (34/148) of the raw water samples. No cysts were detected in finished water samples during the last two years of the FPPE program.

Crockett and Haas (1997), in evaluating Philadelphia's watershed for *Giardia* and *Cryptosporidium*, concluded that information on watershed characteristics was necessary in order

to adequately interpret monitoring data on occurrence of the organisms. By identifying land uses associated with protozoa sources and using factors like runoff during wet weather to set priorities, it should be possible to identify the type of pollution (point or non-point), its general location (immediate or upper regions of the watershed), and whether it occurred daily or only during wet weather.

Crabtree et al. (1996) reported the analyses of water samples from various cisterns in the U.S. Virgin Islands. Over a one-year sampling period, a total 45 samples were analyzed for *Giardia* cysts. The reported average level of *Giardia* was 1.09 cysts/100 L, with a range from <1 to 3.79 cysts/100 L. Of the samples analyzed, 26% were positive for cysts. The cisterns positive for *Giardia* ranged from 18% in January 1993 to 54% in July 1992. These cisterns used roof catchment systems and are obviously open to the air to collect the rain water. They were also described as having a "dark and moist interior," so most may be somewhat covered to reduce the effects of evaporation. However, the investigators described the likelihood that droppings from birds, rodents, and other animals may fall onto the collection areas, and that frogs may also enter and/or live in the cisterns. This represents an unusual type of "surface water," but it is indicative of the potential for the presence of *Giardia* in another type of drinking water source that is used in areas of the Caribbean, as well as other parts of the United States, where rainfall amounts are low and surface water supplies are scarce.

Occurrence data for surface waters have also been reported from European and Asian countries. Karanis et al. (1996a) examined raw river water and filter backwash waters from a treatment plant in Germany. For the raw water samples, 83% (10/12) were positive for *Giardia* cysts at levels of 2 to 103 cysts/100 L (median = 24.5). Similarly, 84% (42/50) of backwash water samples were positive at densities ranging from 3 to 374 cysts/100 L; the median was 22.3 to 55.1, depending upon the method used to process the samples and the sampling depth in the sedimentation basins used to hold the backwash water.

Ahmad et al. (1997) investigated raw and treated waters from two drinking water treatment plants in Selangor, Malaysia. The samples were collected on 10 separate occasions between July, 1994 and January, 1995 and examined for *Giardia* and *Cryptosporidium*. Both treatment plants used the same river as a source and provided conventional treatment. Ninety percent (18/20) of the raw water samples were positive for *Giardia* at levels of 100 to 2,140 cysts/100 L. The two negative samples were collected during periods of high turbidity which may have affected detection of cysts. The authors did not indicate the detection limit for the method used. *Cryptosporidium* oocysts were not detected in the raw water samples and no *Giardia* or *Cryptosporidium* were detected in the treated water samples. No correlation was found between *Giardia* cyst levels in the raw water and fecal coliforms or physical parameters.

Karanis et al (1998) summarized the results of examining raw water, intermediate steps in the treatment process and drinking water samples from six surface water treatment plants in Germany. Sixty-four percent (67/105) of the raw water samples were positive for *Giardia* cysts at an average level of 88.2/100 L (maximum = 1,314/100 L). Twenty percent (30/150) of the samples examined after intermediate steps in the treatment process (e.g., flocculation, filtration) were positive, and the average level was 2.86 cysts/100 L (maximum = 16.8/100 L). About 15% (7/47) of the drinking water samples were positive. These authors had previously reported 84% of backwash samples positive for *Giardia* with a maximum of 374 cysts/100 L (Karanis et al., 1996a). In the most recent study, a single sample of backwash water from an activated carbon filter was examined and found to contain 3,428 cysts/100 L.

Ho and Tam (1998) examined samples over a 13-month period from two Hong Kong rivers for *Giardia*, *E. coli*, and *Cryptosporidium*. Although oocysts were seldom detected in both rivers, high levels of *Giardia* cysts were found: up to 46,880 cysts/100 L in one river and more than 10,000 cysts in the other. The highest cyst levels were found at sampling sites located near the more densely populated areas. No relationship was found between cyst levels and *E. coli* levels in either river. The authors suggested that *Giardia* might be used as an indicator of

sewage treatment plant efficacy and that consideration should be given to including a parasitological indicator of water quality in local river waters.

2. Groundwaters

Hibler (1988) conducted analyses of drinking water samples from various municipal systems, including some from groundwater sources. *Giardia* cysts were found in 19% (16/84) of springs sampled, in 3% (2/63) of wells, and 19% (5/16) of infiltration galleries. His report did not include quantitative data on the cyst levels detected. The author indicated that one of the positive wells was "... essentially an infiltration gallery of the creek ..." which was about 25 feet from the well. The other was a deep well that had been contaminated by priming with contaminated river water.

Lee (1993) reported the contamination of two wells in Pennsylvania with *G. lamblia* by surface streams less than 100 feet from the wells. Information about particulate analyses, water quality analyses, well construction, stream flow, and aquifer characteristics helped demonstrate the contamination source. *G. lamblia* was recovered from all samples collected from the wells.

Hancock et al. (1997) conducted a study involving 463 groundwater samples from 199 sites in 23 states in the United States. Information on aquifer type, geologic setting or well construction details was not submitted with the samples. *Giardia* cysts were found in 6% (12/199) of the sites: 14% (5/35) of the springs, 1% (2/149) of the vertical wells, 36% (4/11) of the horizontal wells, and 25% (1/4) of the infiltration galleries. Of the total of 463 samples analyzed, 23 (5%) were found to contain *Giardia* cysts, with the mean levels in cyst-positive samples being 8/100 L and the median being 2/100 L. The range was 0.1 to 120 cysts/100 L. These water samples were collected by drinking water utilities as part of their routine sampling. Withdrawal rates from the ground water are high and these wells are flushed rapidly. These data

suggest that groundwaters, including some types of springs, and especially groundwater supplies under the influence of surface water, should not be assumed to be free of *Giardia* cysts.

II Occurrence in Soil

No published reports indicating the detection of *Giardia* cysts in soil were found. The wide distribution of cysts in human and animal populations (Chapter III, Section A) indicates that soil is being contaminated with *Giardia* through fecal deposition, irrigation and sewage treatment practices. However, no data are available on cyst levels in soil, survival in soil, or transport through soil matrices. A progress report from the New York State Water Resources Institute Center for the Environment (Cornell University Whole Farm Planning Scientific Support Group, 1993) discussed development and evaluation of a provisional soil sampling protocol and detection method for *Cryptosporidium parvum* oocysts and *Giardia* cysts. The methods were intended to be used to evaluate protozoan prevalence and transport on demonstration dairy farms within a watershed. The investigators concluded that their soil smear/fluorescent antibody method was a rapid method for the detection of oocysts in soil if the numbers of organisms were above the detection limit (about 5×10^3 oocysts/g of soil, wet weight). The authors also suggested that a higher sensitivity automated counting method may be needed for routine counting of oocysts in soil and sediment. No detection limits were indicated for *Giardia* cysts, and no data were presented on occurrence of either cysts or oocysts in the farm soil samples examined.

II Occurrence in Air

No data were found indicating that *Giardia* cysts are released into the air and are transported via the airborne route.

II Occurrence on Surfaces

Cody et al. (1994) developed and evaluated a method for recovering *Giardia* cysts from environmental surfaces, and then field tested the method in six commercial child day-care centers. The method was capable of recovering spiked cysts from Formica® surfaces when they were inoculated with 10 to190 cysts on a surface area of 50 cm^2 or with 10 to 20 cysts/400 cm^2. Cysts were also recovered from stainless steel surfaces inoculated with 20-186 cysts/400 cm^2, but mean recoveries were lower than from Formica® and false negatives were higher. Cysts were not recovered from wood and fiberglass surfaces spiked with 190 cysts/400 cm^2. In the field test, cysts were detected on surfaces in two of the six day-care centers where samples were collected. A total of 53 chairs and tables were examined and two fiberglass chairs (6%) and one Formica® table (2%) surface were positive for *Giardia* cysts. The authors indicated that this was the first reported successful method for examining environmental surfaces for disease-causing protozoan organisms.

II Occurrence in Food

There is a lack of quantitative data on the occurrence of *Giardia* cysts in foods. Although foodborne giardiasis outbreaks (Section F.4, Chapter III) have involved fish, sandwiches, vegetables, fruit and noodle salad, the source of cyst contamination of the food has generally been epidemiologically associated with infected food handlers. In one instance the food had been prepared in the home of women who had a diapered child and a pet rabbit, both positive for *G. lamblia*. Sheep tripe soup was considered to be the vehicle of an outbreak of giardiasis affecting two Turkish families (Karabiber and Aktas, 1991). It was suggested that *Giardia* cysts in deep layers of the tripe were protected from heat inactivation during preparation of the soup.

Barnard and Jackson (1984) discussed methods for detecting *Giardia* cysts in foods and reviewed outbreaks of foodborne giardiasis. They indicated there are no standardized methods to examine foods for *Giardia* but described four techniques originally developed for clinical

specimens that have been adapted to foods. One of these techniques (sedimentation/zinc sulfate flotation) was used by Italian investigators to isolate *Giardia* cysts from lettuce. In that 1968 study, 75% of 64 heads of lettuce collected at random from four markets in Rome, Italy, were found positive for cysts. Barnard and Jackson (1984) indicated this may have been the first reported finding of *Giardia* in vegetables. Oliveira and Germano (1992) found *Giardia* sp. In 4% of lettuce and 10% endive sampled from vegetables traded in S. Paulo, Brazil. Bier (1991) found recovery of seeded *Giardia* cysts from fruits and vegetables using a method employed by the Food and Drug Administration. Improvements are needed in both sampling and analysis.

Rabbani and Islam (1994) found that foodborne outbreaks of giardiasis have been suspected and suggested as early as 1922 and that water, vegetables and other foods were reported contaminated with cysts. They indicated that eating raw or undercooked food because of taste considerations or to conserve heat-sensitive nutrients might increase the risk of spreading *Giardia* through food.

Fayer et al. (1998) examined 360 oysters (*Crassostrea virginica*) collected from six sites in the Chesapeake Bay during May, June, August and September, 1997. Although presumptive *Cryptosporidium* oocysts were identified in either hemocytes or gill washings from oysters collected at all six sites, *Giardia* cysts were not found in any of the samples. The authors indicated that cysts were detected in positive control specimens with the methods used and suggested that failure to detect them in the oysters from the six sites may have been because cysts were not present in the water, not removed by the oysters if they were present, were present below detectable levels or because detection was masked by unknown factors in the hemolymph or gill washings.

II Disease Outbreaks and Endemic Risks

Waterborne giardiasis has been associated with the ingestion of contaminated water from public and private water systems, from untreated and non-potable water sources, and during water recreation and other water-related activities. Both visitors and residents have been affected in outbreaks. In addition to outbreaks, endemic waterborne disease has been reported. Outbreaks have also been traced to ice used for beverages and foods contaminated during their preparation and handling. Person to person transmission has been documented among travelers and in day-care settings, and a high risk of giardiasis has been associated with oral-anal sex among male homosexuals. The relative importance of waterborne giardiasis compared to other modes of transmission of giardiasis has not been well studied, but it has been estimated that perhaps up to 60% of all cases may be waterborne (Bennett et al., 1987).

1. Outbreaks Associated with Drinking Water

The waterborne transmission of *Giardia* was suggested as early as 1946 by an outbreak of amebiasis caused by sewage contamination of the water supply in a Tokyo apartment building; *Giardia* was isolated from 86% of the occupants who had negative stools for *E. histolytica* and experienced diarrhea with abdominal discomfort (Craun, 1990). Waterborne outbreaks of giardiasis were not reported in the United States until 1965, most likely because the pathogenicity of *Giardia* was still being debated. However, it appears in retrospect that a large outbreak of 50,000 cases of illness in Portland, Oregon, in 1954-55 may have been caused by *Giardia* and may possibly have been associated with drinking water (Veazie, 1979). In this outbreak, an unusual prevalence of *G. lamblia* cysts was found in the stools of patients, especially among those with a chronic illness of 14.8 days average duration characterized by abdominal discomfort, diarrhea, loss of appetite, nausea, and weight loss. The first well documented waterborne outbreak of giardiasis in the United States was recognized and investigated primarily because a physician had developed characteristic symptoms of giardiasis after returning from a ski holiday at Aspen, Colorado, in 1965 (Craun, 1990). Fluorescent and

detergent tracers placed in Aspen's sewage system were detected in two of the city's wells, and *Giardia* cysts were isolated from the sewage leaking from sewer mains near the wells.

Although waterborne outbreaks of giardiasis have also been reported in Europe, these outbreaks have been more frequently reported in the United States (Hunter, 1997). Information about waterborne outbreaks of giardiasis reported in the United States during 1965-1977 was previously been summarized (ICAIR, 1984). *Giardia*, the most commonly identified cause of waterborne outbreaks during this period of time, continues to be the most commonly identified cause of outbreaks of waterborne disease in the United States (Craun, 1986; 1990; Moore et al., 1993; 1994; Kramer et al., 1996). Statistics (Craun and Calderon, 1997) published on the causes of waterborne outbreaks in the United States during the period 1971 to 1994 show 740 waterborne outbreaks were reported in community (37%), non-community (40%), individual (11%) water systems, and during recreational activities such as swimming or backpacking when contaminated water is ingested (12%). An etiologic agent was identified in 53% of these reported waterborne outbreaks (Table III-2). Outbreaks were caused by protozoa (20%),bacteria (15%), chemicals (10%), or viruses (8%). *G. lamblia* was the most frequently identified etiologic agent of waterborne outbreaks (17%) during this period.

To be defined as a waterborne outbreak and included in these statistics, epidemiological evidence must implicate water as the probable source of illness. Information about water system deficiencies is usually available, but information about coliform bacteria or *Giardia* in source waters or tap water is not always available. Epidemiological data have been weighted more heavily than water quality data in defining waterborne outbreaks (Kramer et al., 1996).

Table III-2. Summary of Waterborne Outbreaks Reported in the U.S.A., 1971-94*

Etiology	Percent of Outbreaks
Unidentified	47%

Giardia	17%
Bacterial	15%
Chemical	10%
Viral	8%
Other Parasitic	3%
Total	100%

*Includes outbreaks associated with accidental ingestion during water recreation and consumption of non-potable water.

a. Drinking Water Outbreaks in the United States 1965-1996

From 1965 to 1996, 118 outbreaks of giardiasis and 26,305 cases of illness (data compiled by Craun for this document) were associated with the consumption of contaminated drinking water from public and individual water systems in the United States (Table III-3). Cases of illness in these outbreaks include laboratory confirmed cases and persons with symptoms compatible with giardiasis. No deaths were associated with these outbreaks. Most outbreaks (70%) and cases of illness (88%) occurred in community water systems; 22% of the outbreaks and 12% of the cases occurred in non-community water systems. Thirteen additional outbreaks during this period were associated with water recreational and other water-associated activities (discussed in Section G. 2, Chapter III). Waterborne outbreaks were reported in 27 states; five states reported only water recreational-associated outbreaks.

Colorado reported 45 outbreaks associated with drinking water. Pennsylvania reported nine outbreaks, the second highest number. Waterborne outbreak statistics do not provide the actual incidence of waterborne outbreaks or disease and are largely a reflection of surveillance activities of local and state health agencies during the various time periods (Craun, 1986; 1996). Many factors influence the degree to which outbreaks are recognized, investigated, and reported in any single year, including interest in the problem and the capabilities for recognition and investigation at the state and local level (Frost et al., 1996; Berkelman et al., 1994). Improved

surveillance activities have resulted in increased reporting of waterborne outbreaks (Craun, 1986; Foster, 1990; Craun, 1990; Harter et al., 1985; Hopkins et al., 1985). For example, during a three year period of intensive waterborne disease surveillance in Colorado from 1980 to 1983, 18 waterborne outbreaks were reported compared to only six during the previous three year period when a passive surveillance program was in effect (Hopkins et al., 1985). Improved surveillance activities were felt to be responsible for several states reporting large numbers of outbreaks during certain time periods (Craun, 1986). Estimates suggest that in the United States one-half to one-third, or even as few as 10%, of waterborne outbreaks may be detected, investigated, and reported (Craun, 1986; 1996).

Information was recently made available for waterborne outbreaks reported during 1995-1996 (Levy, in press 1998). An outbreak of 10 cases of giardiasis was associated with drinking untreated surface water in Alaska, and 1449 cases were associated with inadequate filtration of a surface water source in a community of 20,000 persons in New York. The outbreak in New York occurred during December 1995 to February 1996 (Hopkins et al., 1998). Heavy rains occurred before the outbreak, and turbidity in filtered water exceeded regulatory limits before and during the outbreak; there was no interruption of chlorination. In 1996, an outbreak of giardiasis was associated with a contaminated wading pool in Florida.

Table III-3. Drinking-Water Related Giardiasis Outbreaks Reported in the U.S.A., 1965 to 1996

Year	Community Water Systems		Non-community Water Systems		Individual Systems & Non-potable Water		Total	
	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases
1965	1	123	0	0	0	0	1	123
1966-8	0	0	0	0	0	0	0	0
1969	0	0	1	19	0	0	1	19
1970	1	34	0	0	0	0	1	34
1971	0	0	0	0	0	0	0	0
1972	1	12	3	112	0	0	3	124
1973	2	52	1	16	1	5	4	73
1974	2	4,878	1	18	1	34	4	4,930
1975	0	0	0	0	1	9	1	9
1976	1	600	2	39	0	0	3	639
1977	2	950	2	62	0	0	4	1,012
1978	2	5,130	1	23	1	18	4	5,171
1979	5	3,789	2	2,120	0	0	7	5,909
1980	7	1,724	0	0	1	6	8	1,730
1981	8	265	2	39	1	7	11	311
1982	9	497	2	60	1	4	12	561
1983	17	2,216	0	0	1	4	18	2,220
1984	3	463	1	400	1	3	5	866
1985	1	703	2	38	0	0	3	741
1986	4	251	1	23	0	0	5	274
1987	2	633	0	0	0	0	2	633
1988	2	262	0	0	0	0	2	262
1989	3	380	1	152	0	0	4	532
1990	1	123	2	42	0	0	3	165
1991	0	0	2	28	0	0	2	28
1992	2	95	0	0	0	0	2	95
1993	2	27	0	0	0	0	2	27
1994	3	358	0	0	0	0	3	358
1995	1	1449	0	0	1	10	2	1459
1996	0	0	0	0	0	0	0	0

Total	***82***	***25,014***	***26***	***3,191***	***10***	***100***	***118***	***28,305***

Waterborne outbreaks of giardiasis have occurred primarily in surface water systems. Statistics available for the period 1971 to 1994 allow for a comparison of etiological agents that were associated with untreated or inadequately treated surface water and groundwater source. An etiologic agent was identified in most outbreaks (66%) caused by inadequately treated surface water but in only 38% of outbreaks caused by inadequately treated groundwater (Table III-4). Most (78%) outbreaks of known etiology in inadequately treated surface water systems were caused by *Giardia*. In untreated or inadequately treated groundwater systems, *Giardia* caused 12% of the outbreaks of known etiology.

Table III-4. Etiology of Waterborne Outbreaks Caused by Contamination of Untreated Water Sources, Inadequate or Interrupted Disinfection, and Ineffective Filtration, U.S.A., 1971-94*

Surface water systems

Etiologic Agent	Outbreaks	Cases of Illness	Hospitalized Cases
Giardia	81	22,171	28
Undetermined	53	20,791	31
Bacterial	10	6,108	30
Viral	6	2,049	0
Chemical	4	268	0
Other Protozoa	3	416,240	4,400
Total	157	467,627	4,489

Groundwater systems

Etiologic Agent	Outbreaks	Cases of Illness	Hospitalized Cases
Undetermined	212	49,351	142
Bacteria	47	8,578	481
Viral	35	8,686	85
Chemical	26	978	19
Giardia	16	336	7
Other Protozoa	6	3,573	8
Total	342	71,502	742

*Outbreaks in surface water and groundwater systems do not include those caused by distribution/storage contamination, recreational activities, or miscellaneous and unknown causes (Unpublished data compiled by G. Craun, 1998).

Craun (1996) computed outbreak rates for community systems using either surface or groundwater sources. Only outbreaks caused by source contamination and treatment inadequacies were considered. These statistically stable rates were used to compare outbreak risks among the different types of water sources and treatment. Community water systems that filter and disinfect surface water experienced 6.3 outbreaks per 1000 systems (95% C.I.=4.2-9.1). Community water systems that used surface water sources with disinfection as the only treatment experienced an outbreak rate of 52.8 per 1000 systems (95% C.I.=40.6-67.6), eight times the rate of outbreaks in filtered surface water systems. Outbreaks that contributed to the high outbreak rates in unfiltered community water systems were caused primarily by *G. lamblia.*

Of the 127 waterborne outbreaks of giardiasis reported during 1971-94, 109 were attributed to drinking water sources and had adequate information to describe the water system deficiency (Table III-5).

Table III-5. Causes of Waterborne Outbreaks of Giardiasis, USA, 1971-94

Water Source, Treatment, or Deficiency	Outbreaks
Surface Water Source:	
Untreated	13
Chlorination Only	51
Filtered (includes outbreaks where filtration was by-passed)	17
Ground Water Source:	
Untreated	8
Chlorination Only	7
Filtration	1
Contamination of Distribution System or Storage	12
Use of Water not Intended for Drinking or Ingestion during Water Recreation or Other Water Activities	14
Insufficient Information	4
Totals	127

Eighty-one (74%) of these outbreaks were caused by inadequate treatment of surface water with the majority occurring in surface water systems that were chlorinated but not filtered. Eighteen (14%) outbreaks occurred in filtered surface water systems when filtration deficiencies were noted or pretreatment or filtration was by-passed. Almost 15% of giardiasis outbreaks occurred in water systems where groundwater sources were inadequately protected and/or treated; 11% of the drinking water-associated outbreaks of giardiasis were attributed to distribution system deficiencies.

Table III-6 presents basic information about all waterborne outbreaks of giardiasis reported in the United States since 1965: state and beginning month of each outbreak, type of water system, deficiencies causing the outbreak, and information available about bacterial water quality, turbidity, and qualitative and quantitative information about *Giardia* cysts in source or treated water. Outbreaks described in the scientific literature since the ICAIR document are also discussed in this section.

A large outbreak of 703 confirmed and 3800 estimated cases of giardiasis occurred during November 1985 to January 1986 in Pittsfield, Massachusetts, where chlorination was the only treatment of surface water (Kent et al., 1988). Illness peaked two weeks after an auxiliary surface water reservoir was placed into service, causing increased complaints of turbid water. An epidemiological study found that giardiasis was higher among residents of areas supplied by the auxiliary reservoir. *Giardia* cysts were detected in water samples from both the auxiliary reservoir and the two other surface water reservoirs. Beavers and muskrats in the vicinity of the reservoirs were found to be infected with *Giardia* and may have contributed to the contamination of the reservoirs. The chlorinator at the auxiliary reservoir also malfunctioned during the entire month of November, but it is not clear that the malfunction contributed to the outbreak, since the chlorine concentrations and contact times which were normally provided were insufficient to inactivate 99.9% of *Giardia* cysts in this water (Craun, 1990). Five days after the episode of turbid water, greater than 5 coliforms/100 mL were found in five of seventeen water samples

from the distribution system. Check samples were taken daily for the next seven days, but apparently no attempt was made at this time to determine the cause of contamination or repair and adjust the chlorinator, as coliforms continued to be found in the check samples.

Table III-6. Outbreaks of Giardiasis Associated with Drinking Water Systems, USA, 1965-96

Location	Date	Cases	Water System[a]	Deficiency	Water Quality	*Giardia* Found[b]
Aspen, CO	Dec. 1965	123	C	Sewage contamination of wells; also used was an unfiltered, disinfected stream source	Coliform contamination of water distribution system	
Lookout Mountain, CO	Aug. 1969	19	NC	Unknown	Unknown	
Idyllwild, CA	May 1970	34	C	Surface water source with filtration and disinfection; filters used intermittently	Positive tap water sample during outbreak (>16 coliforms/100 mL); negative samples before outbreak	
Campground, Boulder, CO	May 1972	28	NC	Unfiltered, chlorinated surface water; interrupted disinfection	Unknown	
Resort, High Co., CO	May 1972	24	NC	Unfiltered, chlorinated surface water; interrupted disinfection	Unknown	
Camp, UT	Sept. 1972	60	NC	Untreated surface water	Unknown	
Park Co., CO	Dec. 1972	12	C	Septic tank seepage into wells; no treatment	Unknown	
Grand Co., CO	July 1973	16	NC	Untreated river water	Unknown	
Farm, TN	Aug. 1973	5	I	Seepage from a pit privy contaminated cistern	Unknown	
Essex Center, VT	Nov. 1973	32	C	Unfiltered, chlorinated surface water; pond	Low coliform count after chlorination	
Danville Green, VT	Dec. 1973	20	C	Unfiltered, chlorinated surface water; pond	Septic tank drainage into pond; no chlorine residual	
Grand Co., CO	June 1974	18	NC	Unfiltered, chlorinated surface water	Unknown	
Meriden, NH	June 1974	78	C	Unfiltered, chlorinated surface water; river	Unknown	

Table III-6. (Cont.) Outbreaks of Giardiasis Associated with Drinking Water Systems, USA, 1965-96

Location	Date	Cases	Water System[a]	Deficiency	Water Quality	*Giardia* Found[b]
Campers, UT	Sept. 1974	34	I	Untreated river water	Fecal coliforms 42/100 mL	
Rome, NY	Nov. 1974	4,800	C	Unfiltered, chlorinated surface water; lake	527 tap samples Nov–June negative for coliforms; Feb samples: 20, 20, 30, and 40 coliforms/100 mL	Yes
Campers, ID	Sept. 1975	9	I	Untreated surface water	Unknown	
Grand Co., CO	Feb. 1976	12	NC	Untreated river water	23 coliforms/100 mL	
Camas, WA	May 1976	600	C	Wells and river water treated by pressure filtration and chlorination; deficiencies in operation of filters and disinfection	Coliforms found but treated water met coliform MCL; turbidity was 4.2 NTU.	Yes
Camp, Estes Park, CO	June 1976	27	NC	Unfiltered, chlorinated surface water; lake	Water source contained <0.5 fecal coliforms/100 mL.	Yes
Berlin, NH	April 1977	750	C	River water sources with conventional and pressure filtration and chlorination; deficiencies in construction of rapid sand filter and operation of pressure filter	Routine distribution system samples were consistently negative and met MCL.	Yes
Resort, Wasatch Co.,UT	June 1977	7	NC	Untreated well water under the influence of surface water (river)	14-110 coliforms/100 mL; turbidity=1.4 NTU.	
Hotel, Glacier Park, MT	July 1977	55	NC	Use of untreated surface water	Unknown	
W. Sulfur Springs, MT	July 1977	200	C	Unfiltered, chlorinated surface water	Unknown	
Vail, CO	Mar. 1978	5, 000	C	Inadequate filtration of river water	14 coliforms/100 mL, MCL not exceeded; turbid.=3 NTU	Yes

Table III-6. (Cont.) Outbreaks of Giardiasis Associated with Drinking Water Systems, USA, 1965-96

Location	Date	Cases	Water System[a]	Deficiency	Water Quality	*Giardia* Found[b]
Picnic, UT	July 1978	18	I	Untreated irrigation water	Not Reported	
Camp, WA	Aug. 1978	23	NC	Untreated surface water	Not Reported	Yes
Town, NY	Nov. 1978	130	C	Unfiltered, chlorinated surface water	Not Reported	
CA	Feb. 1979	120	C	Unfiltered, chlorinated surface water	Not Reported	
Town, NH	April 1979	50	C	Unfiltered, chlorinated surface water	Not Reported	
Campground, AZ	June 1979	2,000	NC	Distribution system deficiency; well water	9 fecal coliforms/100 mL	
Town, CO	June 1979	53	C	Inadequate filtration of river water	Not Reported	Yes
Bradford, PA	Sept. 1979	3,500	C	Unfiltered, chlorinated surface water	High coliforms count; turbidity=10 NTU	Yes
Town, OR	Oct. 1979	66	C	Unfiltered, chlorinated surface water	Not Reported	Yes
Town, OR	Dec. 1979	120	NC	Unfiltered, chlorinated river water	Not Reported	
Town, WA	Jan. 1980	79	C	Untreated surface water	Heavy rains; high turbidity	
Town, WA	Mar. 1980	578	C	Inadequate filtration of river water	Not Reported	
Apart. Bld., PA	Mar. 1980	15	C	Treatment deficiency; spring	Not Reported	
Backpackers, WA	Apr. 1980	6	I	Untreated stream water	Not Reported	
Red Lodge, MT	June 1980	780	C	Unfiltered, chlorinated stream water	Not Reported	No
Town, OR	June 1980	63	C	Unfiltered, chlorinated stream water	No coliforms detected	
Town, Alaska	Sept. 1980	189	C	Distribution system; cross-connection	>2400 fecal coliforms/100 mL	

Table III-6. (Cont.) Outbreaks of Giardiasis Associated with Drinking Water Systems, USA, 1965-96

Location	Date	Cases	Water System[a]	Deficiency	Water Quality	*Giardia* Found[b]
WA	Sept. 1980	20	C	Distribution system; cross-connection	Not Reported	
Town, CO	June 1981	8	C	Inadequate filtration of stream water	Not Reported	
Town, CO	July 1981	30	C	Unfiltered, chlorinated stream water	Not Reported	
Town, CO	Aug. 1981	110	C	Treatment deficiency; creek	Not Reported	
CO	Sept. 1981	32	C	Treatment deficiency; creek	No coliforms detected	Yes
Motel, WI	Sept. 1981	25	C	Insufficient data; well water	Not Reported	
Town, VT	Oct. 1981	22	C	Untreated surface water; spring	Not Reported	
Hikers, CO	Oct. 1981	7	I	Untreated river water	Not Reported	
Factory, FL	Oct. 1981	7	NC	Water not intended for drinking	Not Reported	
Ski area, CO	Nov. 1981	38	C	Inadequate filtration; stream	Fecal coliforms found	Yes
Town, CO	Dec. 1981	14	C	Interrupted chlorination; unfiltered spring	2-27 fecal coliforms/100 mL	
Town, CO	Dec. 1981	18	C	Inadequate filtration; stream	Not Reported	
Ski resort, CO	Jan. 1982	10	C	Inadequate filtration; stream	Not Reported	
Ski resort, CO	Feb. 1982	17	C	Inadequate filtration; stream	Not Reported	
Ski resort, CO	Mar. 1982	4	C	Inadequate filtration; stream	Not Reported	
Ski resort, CO	April 1982	8	C	Insufficient data	Not Reported	
Home, VA	April 1982	4	I	Seepage of sewage into untreated well	Not Reported	
CO	July 1982	72	C	Unfiltered, chlorinated stream water	Not Reported	

Table III-6. (Cont.) Outbreaks of Giardiasis Associated with Drinking Water Systems, USA, 1965-96

Location	Date	Cases	Water System[a]	Deficiency	Water Quality	*Giardia* Found[b]
Ranch resort, CO	Aug. 1982	28	NC	Treatment deficiency; river	Not Reported	
Reno, NV	Aug. 1982	342	C	Unfiltered, chlorinated surface water	No coliforms detected	0.26/100 L
Ski resort, CO	Sept. 1982	32	NC	Interrupted chlorination of stream water	16-69 coliforms/100 mL	Yes
Community, OR	Sept. 1982	9	C	Unfiltered, chlorinated surface water	Not Reported	
Community, NH	Oct. 1982	13	C	Insufficient data	Not Reported	
Community, VT	Oct. 1982	22	C	Untreated ground water; springs	2 coliforms/100 mL	
Community, CO	Jan. 1983	4	C	Untreated surface water; river	No coliforms detected	Yes
Community, CO	Jan. 1983	11	C	Untreated surface water; river	No coliforms detected	
Community, CO	Jan. 1983	17	C	Inadequate filtration of river water	No coliforms detected	Yes
Community, UT	Jan. 1983	41	C	Contamination of water main under repair	No coliforms detected	
Trailer park, FL	Mar. 1983	3	C	Inadequate chlorination; wells	Not Reported	
CO	May 1983	11	C	Inadequate filtration of lake water	No coliforms detected	
Community, CO	May 1983	10	C	Filtration bypassed; stream	No coliforms detected	
Community, NH	May 1983	7	C	Unfiltered, chlorinated surface water	Not Reported	
Household, VA	June 1983	4	I	Untreated well water	9.2 fecal coliforms/100 mL	
Community, MT	July 1983	100	C	Unfiltered, chlorinated surface water	Not Reported	
Community, NM	Aug. 1983	100	C	Unfiltered, chlorinated surface water	Not Reported	
Community, UT	Aug. 1983	1,272	C	Contamination of broken water main	Coliforms in 3 samples	

Table III-6. (Cont.) Outbreaks of Giardiasis Associated with Drinking Water Systems, USA, 1965-96

Location	Date	Cases	Water System[a]	Deficiency	Water Quality	*Giardia* Found[b]
Community, CO	Oct. 1983	11	C	Coss-connection	106 coliforms/100 mL	
16 commun., PA	Oct. 1983	366	C	Unfiltered, chlorinated surface water	Not Reported	Yes
Community, PA	Oct. 1983	135	C	Unfiltered, chlorinated surface water	Not Reported	Yes
CO	Nov 1983	13	C	Inadequate filtration of surface water	Not Reported	
Community, ID	Nov. 1983	44	C	Unfiltered, chlorinated surface water; reservoir	36 coliforms/100 mL	
Community, ID	Nov. 1983	71	C	Unfiltered, chlorinated surface water; river	No coliforms detected	
Community, PA	Feb. 1984	298	C	Inadequate filtration of surface water; river	Not Reported	
Ski resort, CO	Mar. 1984	400	NC	Unfiltered, chlorinated surface water; pond	Not Reported	265/100 L
Community, OR	July 1984	42	C	Inadequate filtration of surface water; river	Not Reported	0.02/100 L
Camp, AK	Sept. 1984	3	I	Untreated surface water	Not Reported	
Community, AK	Oct. 1984	123	C	Unfiltered, chlorinated surface water; reservoir	Not Reported	
Factory, NY	Feb. 1985	6	NC	Distribution system; cross-connection	24 coliforms/100 mL	
Resort, VA	April 1985	32	NC	Inadequate chlorination; spring	Not Reported	
Pittsfield, MA	Nov. 1985	703	C	Interrupted chlorination; unfiltered water from reservoir	>5 & 8-41 coliforms/100 mL; period of high turbidity	0.02-0.07/100 L
Trailer park, VT	Jan. 1986	68	C	Unfiltered, chlorinated surface water; river	Water source >20,000 coliforms/100 mL	Yes
Prison, CA	Apr. 1986	127	C	Leaking, broken water lines	Not Reported	
Resort, CO	Aug. 1986	23	NC	Interrupted chlorination; well	12 coliforms/100 mL	

Table III-6. (Cont.) Outbreaks of Giardiasis Associated with Drinking Water Systems, USA, 1965-96

Location	Date	Cases	Water System[a]	Deficiency	Water Quality	*Giardia* Found[b]
Community, ME	Nov. 1986	12	C	Unfiltered, chlorinated surface water; river	1 of 16 samples positive	
City, NY	Nov. 1986	44	C	Unfiltered, chlorinated surface water; lake	Not Reported	0.1-0.3/100 L
Community, PA	April 1987	513	C	Unfiltered, chlorinated surface water; river	Turbidity >1 NTU	
Community, CT	July 1987	120	C	Distribution system; cross-connection	80-800 coliforms/100 mL	
Resort, CO	Feb. 1988	90	C	Unfiltered, chlorinated surface water; river	Not Reported	<0.01/100 L
Community, PA	July 1988	172	C	Unfiltered, chlorinated surface water; lake	Not Reported	
Community, CO	Feb. 1989	19	C	Unfiltered, chlorinated surface water; river	No coliforms detected	
Community, NY	April 1989	308	C	Unfiltered, chlorinated surface water; reservoir	Not Reported	0.02/100 L
Prison, NY	June 1989	152	NC	Unfiltered, chlorinated surface water; reservoir	Not Reported	
Community, NY	July 1989	53	C	Unfiltered, chlorinated surface water; lake	Not Reported	<0.01/100 L
Lodge, AK	Mar. 1990	18	NC	Untreated surface water; river	Not Reported	
Resort, VT	Mar. 1990	24	NC	Unfiltered, chlorinated surface water; lake	No coliforms detected	
Community, CO	Aug. 1990	123	C	Inadequate chlorination of spring; surface water influence	35-200 coliforms/100 mL	Yes
Park, CA	July 1991	15	NC	Cross-connection with raw water; spring	No coliforms detected	
Park, PA	Sept. 1991	13	NC	Inadequate chlorination of well	No coliforms detected	
Trailer park, CO	Mar. 1992	15	C	Untreated ground water; well	1 of 2 samples positive	
Community; NV	Mar. 1992	80	C	Unfiltered, chlorinated surface water; lake	No coliforms detected	50/100 L
Trailer park, PA	Jan. 1993	20	C	Inadequate filtration of well	Not Reported	

Table III-6. (Cont.) Outbreaks of Giardiasis Associated with Drinking Water Systems, USA, 1965-96

Location	Date	Cases	Water System[a]	Deficiency	Water Quality	*Giardia* Found[b]
Subdivision, SD	Sept. 1993	7	C	Untreated well in fissured rock	Fecal, total coliforms detect.	32/100 L
Prison, TN	Mar. 1994	304	C	Cross-connection	No coliforms detected	580/ L
Community, NH	May 1994	18	C	Unfiltered, chlorinated surface water; reservoir	No coliforms detected	Yes
Community, NH	May 1994	36	C	Unfiltered surface water; lake	Coliforms detected	No
AK	Aug. 1995	10	I	Unfiltered surface water		
City, NY	Dec. 1995	1449	C	Filtered surface water; lake	Turbidity exceeded MCL	No

*Data compiled by G. Craun, 1998.

[a] C - Community Water System; NC - Non-community Water System; I - Individual Water System Including Personal Use of Non-potable Water Sources

[b] Yes/No - *Giardia* Cysts Found in Source Water or Distribution System; blank - No Samples Collected or Reported

An outbreak of 342 confirmed cases of giardiasis in Pennsylvania during December 1983 and January 1984 is important because it occurred in a surface water system with conventional filtration that routinely met water quality standards (Akin and Jakubowski, 1986). Operational deficiencies were found. An unusually high demand for water had left an insufficient volume of water for filter backwashing, and turbidity breakthrough occurred in the filters because of longer filter runs. A free chlorine residual of 1.0 to 1.3 mg/l was maintained, and the system met coliform standards; however, turbidity increased to 2.80 NTU (a weekly average). Hopkins and Juranek (1991) reported an outbreak among university students and staff on a geology field course in Colorado in June 1983 where the risk of stool positivity was strongly related to the amount of untreated water consumed

Several outbreaks were caused by contamination of water mains through cross-connections, damage of mains, and repair of mains. The largest outbreak of this type, 2000 cases at a private campground in Arizona, occurred when sewage-contaminated water entered the drinking water system through a direct cross-connection between the potable water system and a pipe carrying sewage effluent for irrigation (Starko et al., 1986; Craun, 1986). In Tooele, Utah, 1272 cases occurred when contamination entered a water transmission line which had been damaged by mud slides and flooding due to heavy rains; routine water samples were positive for coliforms prior to this outbreak. (MMWR, 1983). Contaminated water during the repair of a water main was identified as the cause of 41 cases of giardiasis in another outbreak which occurred in Utah (Craun, 1986).

A waterborne giardiasis outbreak at Aspen Highlands, Colorado, in November 1981 affected a small number of people but is important because a clear dose-response relationship was found for water consumption and clinical illness and it offers evidence of acquired immunity to *Giardia* (Istre et al., 1984). An attack rate of 42% was found among persons who drank six or more glasses of water per day. Residents who lived in the area for more than two years had a

lower attack rate for illness than short-term residents. *Giardia* cysts were isolated from raw and treated water samples, and beavers were suspected as the source of contamination.

Vogt et al. (1984) investigated an outbreak in a small Vermont community in December 1981. A serological survey found people who drank the town water had a significantly higher antibody titer to *G. lamblia* than those who had not. *G. lamblia* was identified from only four cases. The community's water supply source was an unprotected, unfiltered, and unchlorinated spring. Birkhead et al. (1989) investigated an outbreak of giardiasis that affected 37 (30%) residents of a trailer park in rural Vermont in 1986. An increased risk of disease was associated with increased water consumption from the trailer park's water system, a chlorinated, unfiltered surface water supply. *Giardia* cysts were found in the water. Convalescent sera from 24 ill residents and from 20 nonresidents were tested by enzyme-linked immunosorbent assay (ELISA) for immunoglobulin G (IgG), IgM, and IgA antibodies to *G. lamblia*. Higher IgA and IgG antibody levels were detected in the ill residents compared to nonresident controls. Nine ill residents had a higher median level of IgA antibody but not of IgG or IgM than 15 healthy residents. In addition, IgA antibody levels for *G. lamblia* were higher in those who consumed tap water than in those who did not. The authors suggested that elevated IgA antibody to *G. lamblia* may effectively determine exposure to cyst contaminated water and subsequent illness during waterborne outbreaks.

b. Waterborne Outbreaks in Canada

Allison (1984) discussed a giardiasis outbreak in a Saskatchewan municipality in 1982 where water may have been the source of infection and the need for better disease surveillance. Several waterborne outbreaks were reported in British Columbia where most municipal water systems are not filtered and, in some instances, not disinfected (Isaac-Renton, 1994a). In Canada, suspected waterborne outbreaks occurred at Banff and Edmonton, Alberta (Wallis et al., 1986). Over 800 people were infected during the Edmonton outbreak. In the Banff outbreak,

infected beavers were found in the creek which supplied the town with water (Wilson et al., 1982). Three documented waterborne outbreaks of giardiasis occurred within five years in British Columbia (Isaac-Renton et al., 1987); one was reported at 100 Mile House (Wallis et al., 1986). All water systems used surface water without filtration, and *Giardia*-positive beavers were found in the water sources. An estimated 3,125 cases of giardiasis occurred in a waterborne outbreak in Penticton, British Columbia from June to August 1986 (Moorehead et al., 1990). The city obtained water from a creek, lake and well. The creek water was very turbid, and although no *Giardia* cysts were isolated from water, beavers trapped in the creek were stool positive. Two muskrats trapped from the lake were also positive.

Isaac-Renton et al. (1993) investigated an outbreak of waterborne giardiasis in Creston, British Columbia, where 124 laboratory-confirmed cases were identified during an eight-week period. This was the second outbreak in this town (Isaac-Renton et al., 1994b). A previous waterborne outbreak of giardiasis had occurred five years earlier but had not caused the small rural town to initiate treatment of the drinking water nor change their water source. An isolate of the outbreak-associated *Giardia* cysts was obtained from the contaminated drinking water, and the antigen from this strain was utilized in the serological testing. Sera from symptomatic and asymptomatic residents were tested by ELISA which positively identified 84% of the 124 laboratory-confirmed cases. There was greater success in identifying elevated anti-*Giardia* IgG levels compared with identifications of elevated levels of IgA or IgM. Residents who had been infected during the first outbreak were significantly less likely to be infected during the second outbreak suggesting an acquired immunity to giardiasis that may last for at least five years. Of 54 laboratory-confirmed cases from the first outbreak, only 4% were infected during the second outbreak, whereas of 57 residents who had moved to the town after the first outbreak, 68% were infected.

c. Waterborne Outbreaks in Europe

The first recognized waterborne outbreak of giardiasis in the United Kingdom occurred in 1985; 108 laboratory-confirmed cases were reported. (Jephcott et al., 1986). Neither coliforms nor *Giardia* were detected in water samples collected from the distribution system of the filtered and disinfected water system. The epidemiologic investigation found a strong association between illness and consumption of water during a time when water mains were repaired. It was suspected that contamination occurred either during the repairs or from backsiphonage from pressure changes associated with the repair. Neringer et al. (1987) reported the first recognized outbreak of giardiasis in Western Europe. The outbreak occurred in Mjovik, Sweden, and had multiple etiologies. During the first few days after sewage contamination of the village well, 76% of the population became ill with gastroenteritis with at least 56 cases of giardiasis occurring several weeks later. An unusual outbreak affected four people in Scotland in June 1990 when a roof-top storage tank was deliberately contaminated with human feces (Ramsay and Marsh, 1990). *Giardia* cysts were found in water from the tank.

One of the largest waterborne giardiasis outbreaks in Europe affected more than 3000 individuals at a ski resort in Sweden during Christmas holidays in 1986 after the overflow of sewage into the drinking water system (Ljungstrom and Castor, 1992). Serum samples were collected from 352 exposed and 428 healthy, unexposed persons. Of the unexposed persons 10% had either IgG or IgA, or both antibodies. Of those exposed, 41% had anti-*Giardia* antibodies. IgG was present in 60% and IgA in 28% of those who had *Giardia* in their stool. In those who were stool negative, 20% and 4% had IgG and IgA. More sera were positive (66% versus 49%) when collection was delayed for three weeks after onset of illness. These results emphasize the importance of identifying infected asymptomatic persons who are not *Giardia*-positive stool microscopy. In outbreak investigations, the selection of cases and controls has been based on symptoms or stool positivity, and this may have caused misclassification bias.

2. Outbreaks Associated with Recreational Water

Because of its low infectious dose, *Giardia* can be transmitted via the accidental ingestion of relatively small volumes of contaminated water while swimming. Fourteen swimming-associated outbreaks and 637 cases of giardiasis were reported in the United States during 1982-1996 and are summarized in Table III-7 (unpublished data compiled by Craun, 1998).

Table III-7 Swimming-Associated Giardiasis Outbreaks Reported 1982-96, U.S.A.

State	Year	Cases	Location	Additional Information
Washington	1982	78	swimming pool	
Illinois	1985	15	swimming pool	fecal contamination
New Jersey	1985	9	indoor pool	
Maryland	1987	266	swimming pool	inadequate chlorination
Maryland	1988	34	swimming pool	
Georgia	1991	9	swimming pool	day-care center
Georgia	1991	7	swimming pool	day-care center
Maryland	1991	14	pool	park; fecal contamination
Washington	1991	4	swimming lake	wild animals near lake
Maryland	1993	12	swimming lake	
New Jersey	1993	43	swimming pond	met water quality limits
Washington	1993	6	river	
Indiana	1994	80	swimming pool	filter malfunction
Florida	1996	60	wading pool	

Relatively few recreational water outbreaks of giardiasis have been described in the literature. In an infant and toddler swim class in Washington State, 71 participants were found to have *Giardia*- positive stools (Harter et al., 1984). Investigation found high turbidity and low chlorine levels were an occasional problem at the pool, and fecal accidents were often reported during the class. Nine cases of giardiasis were identified in people who had been swimming at a pool in New Jersey during one day in September 1985 when a handicapped child had a fecal

accident (Porter et al., 1988). The handicapped child and eight others in a group of twenty were found stool positive. At a hotel in Manitoba, Canada, 59 of 107 guests were reported ill with giardiasis (Greensmith et al., 1988). An association was found between illness and using the water slide on one of three days and swallowing pool water.

3. Outbreaks Associated with Non-potable Water

Outbreaks and illness have also been associated with consumption of untreated water while camping, backpacking, and hiking, from individual water supplies, and from other sources Craun, 1996). For example, an outbreak of 12 cases of giardiasis was reported in 1982 among a group of New York City police and fire fighter divers in the Hudson River. Nine outbreaks and 116 cases of giardiasis were reported in the United States due to use of contaminated water from non-potable and individual water systems. These outbreaks are the least likely to be detected and investigated and thus, do not reflect the incidence of outbreaks among persons using untreated surface water. However, they illustrate the high risk associated with consumption of untreated water. Two outbreaks were associated with use of contaminated, untreated well water at homes without public water supplies, and one outbreak occurred on a farm after contamination of cistern water from a pit privy. An outbreak of 18 cases occurred in Utah in 1978 when persons on a picnic mistakenly drank from a tap that provided irrigation water. Five outbreaks were associated with the use of untreated surface water during hiking, backpacking, or camping.

4. Endemic Waterborne Giardiasis

a. Drinking Water

Because of the low infectious dose and ubiquitous nature of the sources of cysts in many drinking water supplies, it is possible that sporadic cases of waterborne infection might occur in marginally treated water systems, and that these cases of giardiasis would not be recognized as an

outbreak. Epidemiologic studies, however, must be specifically designed to assess endemic waterborne disease risks. Craun (1997) evaluated epidemiological studies where endemic giardiasis was found to be associated with the consumption of untreated or inadequately treated drinking water. Studies in Colorado (Wright et al., 1977), Minnesota (Weiss et al., 1977), Washington (Harter et al., 1982; Frost et al., 1983), New Hampshire (Chute et al., 1985, 1987; Dennis et al. 1993), Utah (Laxter, 1985), and Vermont (Birkhead and Vogt, 1989) have suggested that consumption of untreated drinking water may be an important cause of endemic infection and illness in the United States. A 1973 survey of 256 Colorado residents having *Giardia*-positive stools, when compared to 256 controls matched by age, gender, race, and place of residence, showed a higher proportion of cases among those who visited Colorado mountains (69% vs. 47%), camped overnight (38% vs. 18%), and drank untreated mountain water (50% vs. 17%). A 1975 survey of 78 Minnesota residents with *Giardia*-positive stools and no history of recent foreign travel found that 63% had consumed untreated water during the period of study. Unfortunately, an appropriate control group was not included for comparison. In a case-control study of 349 Washington State residents having *Giardia*-positive stools during July 1978 to March 1980 and 349 controls matched by age and gender, Frost et al. (1996) found consumption of untreated water, nursery school exposure for children, and foreign travel to developing countries to be associated with higher risk of acquiring *Giardia* infection. A survey of intestinal parasites conducted in two Washington counties found a 7% *Giardia* prevalence among 518 children, one to three years of age (Harter et al., 1983). No difference in the prevalence of infection was found for source (surface or well) of drinking water, but a higher risk was associated with use of unfiltered surface water. Ten of 175 (7%) children residing in homes using unfiltered surface water were found to be infected with *Giardia* compared with only one of 37 (3%) children residing in a home using filtered surface water. An increased prevalence of infection was also found in children who had a history of drinking untreated surface water from streams or lakes during recreational activities.

Results of a case-control study of 171 giardiasis patients in New Hampshire during January, 1977 to June, 1984 and 684 controls matched by age and gender found an increased risk of acquiring giardiasis was associated with travel outside the United States, family member diagnosed giardiasis, family member in a day-care program, camping, and use of a shallow well or surface water for individual, household water supply (Chute et al., 1985, 1987). Persons who used shallow well or surface water sources for their household water supply had twice the risk of giardiasis compared to persons who used any other water source, either drilled well or municipal. A higher risk of giardiasis was associated with the household use of shallow well or surface water sources compared to use of municipal water sources. Significant risk factors found in a study of 273 cases and 375 matched controls during 1984 and 1985 in New Hampshire (Dennis et al. 1993) were: drinking water from shallow wells, contact with a person in day care, and swimming in a lake, pond, stream or river. A survey of 383 Utah National Guard members showed that 15% had symptoms suggestive of giardiasis and that the guardsmen were at risk of contracting giardiasis by drinking contaminated water during field exercises in the state (Laxter, 1985); 62% of the men who had symptoms drank untreated water from lakes, streams, and a cattle watering trough.

Birkhead and Vogt (1989) studied risk factors among 1211 cases of laboratory-confirmed giardiasis that were not associated with outbreaks in Vermont and found increased relative risks (RR) of giardiasis for persons using municipal surface water systems without filtration (RR=1.9) or well water (RR=1.8) and persons using private water systems (RR=2.2). The average annual incidence rate of giardiasis was found to lowest in populations using municipal surface water systems with filtration (15.1/100,000) compared populations using municipal surface water systems without filtration (28.6/100,000), populations using municipal well water (26.8/100,000), persons using private water systems (32.8/100,000).

Fraser and Cooke (1991) conducted an epidemiological study in Dunedin, New Zealand, to investigate the risk of endemic giardiasis in a part of the city where water was mechanically

microstrained through a 23 μm screen, chlorinated and fluoridated. A three-fold increased relative risk for giardiasis was found for persons using this water compared to persons in another part of the city where water was treated by coagulation/flocculation and direct dual media filtration (anthracite and sand).

A case-control study in Vancouver, a city with a population of 1.4 million served by an unfiltered but chlorinated supply, found no increased risks of giardiasis associated with drinking water (Mathias et al., 1992). Risk factors for giardiasis were travel abroad and travel elsewhere within British Columbia. Another case-control study elsewhere in British Columbia, however, found that drinking water was the most important risk factor for laboratory-confirmed giardiasis (Isaac-Renton and Philon, 1992). Persons who drank unchlorinated and unfiltered surface water were at a much higher risk compared to those who drank well-water. There was little difference in giardiasis risk, however, for people who drank either chlorinated, unfiltered surface water and unchlorinated, unfiltered surface water. For persons who traveled to rural areas of British Columbia, drinking local tap-water was identified as a risk factor for giardiasis (Isaac-Renton and Philon, 1992).

In a year-long longitudinal study, Isaac-Renton et al. (1996) assessed *Giardia* cyst levels and parasite viability in the drinking water of two British Columbia communities From 69% of the sample locations, 64% of source water samples were found to be cyst-positive. In one community, 77% of water samples of treated water were cyst-positive; in the other, 98% of samples were cyst-positive. In the overall survey and surveys for each community, decreased *Giardia* cyst levels and decreased viability, based on infectivity testing in the Mongolian gerbil model, were found in chlorinated water samples compared with their respective source water samples. Assays in which the gerbils were inoculated orally by gavage found 0% infectivity for the cysts. Low-level endemic waterborne transmission, however, was suggested by results of a health survey. Compared to a nearby community that obtained water from a protected watershed,

both communities had an increased seroprevalence and prevalence of laboratory-confirmed cases.

The consistency of the findings in these studies strongly suggests that the risk of giardiasis is high among persons who consume untreated water and that in the United States, Canada, and New Zealand, endemic risks of waterborne giardiasis are high among populations that consume unfiltered surface water. However, in some developing countries the endemic waterborne giardiasis risk may not be as significant. Esrey et al. (1989) investigated the relationship between the presence of *Giardia* infection in pre-school children and drinking water in Lesotho, South Africa. Results of this study found that in this population personal hygiene and person to person transmission were more important than waterborne transmission.

b. Water Recreation and Other Water Sources

Epidemiological studies of endemic waterborne giardiasis have also identified swimming-associated risks. Engaging in recreational water activities was found to be a risk factor for giardiasis among travelers to rural areas of British Columbia (Isaac-Renton and Philon, 1992). In a case-control epidemiological study in New Hampshire (Dennis et al., 1993) found that swimming in a lake, pond, stream or river was among the several important risk factors for giardiasis. A survey (Harter et al., 1982) in Washington also found an increased prevalence of infection among children who had a history of drinking untreated surface water from streams or lakes during recreational activities. In a study of endemic giardiasis in the Canterbury area of New Zealand, contact with sewage and travel abroad were the most significant risk factors for giardiasis (Hunter, 1998). A case-control study of 74 cases and 108 matched controls from July 1992 to May 1993 in the counties of Somerset and Avon in England found that drinking potentially contaminated water and swimming were significant risk factors (Gray et al., 1994).

5. Foodborne Outbreaks

Outbreaks can occur when food becomes contaminated, but few well-documented foodborne outbreaks of giardiasis have been reported. Rabbani and Islam (1994) subsequently reviewed foodborne giardiasis and concluded that it is rare in developed countries but that food can be an important vehicle of transmission in areas where food hygiene is poor and a significant proportion of the population is infected. They found that foodborne outbreaks of giardiasis have been suspected and suggested as early as 1922.

Epidemiological investigation of a giardiasis outbreak among employees of a school in Minnesota in 1979 found two food items statistically associated with illness, home-canned salmon and cream cheese dip (ICAIR, 1984). The wife of one employee had diapered her 12-month-old grandson just prior to preparing the salmon and touched the salmon by hand before it was given to the employees. Although she was free of *Giardia* symptoms, the grandson's stools were positive for *Giardia*. Peterson et al. (1988) described a foodborne outbreak among 16 attendees at a picnic in rural Connecticut. Food was prepared and brought to the picnic by seven family groups and a neighbor who did not attend. This neighbor prepared a cold noodle salad which was implicated as the vehicle of infection. The salad preparer, who was symptomatic one day after making the salad, could have been excreting *Giardia* cysts the day she made the salad and could have contaminated it while mixing with her hands.

In 1986, a giardiasis outbreak occurred in a nursing home in Minnesota; 73 residents or employees of the home and 15 children participating in day care at the home became ill (White et al., 1989). The mean age of resident cases was 80 years and their mean duration of diarrhea was 16 days. Epidemiological investigation implicated both foodborne and person to person transmission. An association was found between sandwich consumption and illness in nursing home staff, and there was a significant lack of illness among residents who consumed only a pureed diet that was cooked before serving. An outbreak of 10 cases of giardiasis occurred among 25 persons attending a family party in New Jersey in 1986 (Porter et al., 1990). Epidemiological evidence implicated fruit salad that had been prepared in the home of women

who had a diapered child and a pet rabbit, both positive for *G. lamblia*. Nine cases had onset of symptoms 6 to 12 days following the party.

The first reported common-source outbreak of giardiasis traced to a commercial food establishment occurred at a restaurant in central Washington State in 1990 (Quick et al., 1992). Twenty-seven of 36 (75%) persons who had attended a meeting and eaten at the restuarant became ill. Onset of illness occurred 2-19 days after the meeting and illness lasted from 7 to 28 days (median = 18 days). One patient was hospitalized. No single food or beverage was statistically associated with illness, but 26 of the 27 ill persons drank ice water. Although contaminated water was felt to be an unlikely source of infection, the ice could have become contaminated during handling at the restaurant. The restaurant was served by a community water system that obtained water from 18 untreated wells; routine water samples were negative for coliform bacteria. Ice for beverages had been served by an employee who had an asymptomatic *Giardia* infection and an employee who had a *Giardia*-infected child still in diapers. Either food handler could have transferred *Giardia* cysts from their hands to the beverage ice either directly or via the ice scoop. The plausibility of ice as the vehicle of infection is supported by an earlier outbreak in Canada in which ice was suspected and by evidence that surface contamination may be the only mechanism by which ice can serve as a vehicle for *Giardia* transmission (Quick et al., 1992). A similar mode of transmission was suggested in two previous foodborne outbreaks of giardiasis (ICAIR, 1984; Porter et al, 1990). Mintz et al. (1993) describe an outbreak of giardiasis associated with an insurance company cafeteria. A case-control study of 26 sick employees and 162 well employees suggested the probable vehicle of infection was raw, sliced vegetables served in the employee cafeteria. The sliced vegetables had been prepared by a food handler who was infected with *G. lamblia*. The median duration of diarrhea in this outbreak was 35 days.

Foodborne outbreaks of giardiasis have been reported in the United States; small outbreaks have occurred because ice used for beverages and foods were accidently contaminated

by food service workers. In five reported foodborne outbreaks, cold foods such as salmon, raw vegetables, noodle salad, fruit salad, and sandwiches were implicated as vehicles of infection. Ensuring good hygienic practices among food service workers, including hand washing, washing vegetables, and using gloves and clean utensils, are important in preventing foodborne outbreaks of giardiasis (Quick et al., 1992; Mintz et al., 1993).

Although giardiasis outbreaks may often be unrecognized because of the long incubation period and large number of asymptomatic infections, restaurant-associated transmission of *G. lamblia* does not appear to be a significant public health problem (Quick et al., 1992). Reports of parasitological screening studies of food service workers in Panama and Algeria have revealed high rates of asymptomatic infection but no evidence of outbreaks (Quick et al., 1992). Eating raw or undercooked food because of taste considerations or to conserve heat-sensitive nutrients might increase the risk of spreading *Giardia* through food (Rabbani and Islam, 1994). Sheep tripe soup was considered to be the vehicle of an outbreak of giardiasis affecting two Turkish families (Karabiber and Aktas, 1991). It was suggested that *Giardia* cysts in deep layers of the tripe were protected from heat inactivation during preparation of the soup.

6. Travelers

Travelers' diarrhea can be caused by exposure to a number of bacterial, viral, and proptozoan pahogens, including *Giardia* (ICAIR, 1984). Although giardiasis probably accounts for less than 5% of traverlers' diarrhea, high attack rates have been reported in Europeans and North Americians traveling to certain areas of the world (Farthing, 1996). Reports of giardiasis in travelers first appeared in 1970 as outbreaks occurred among travelers to the Soviet Union, especially St. Petersburg (ICAIR, 1984). In the 1970's, outbreaks of giardiasis were reported among a group of travelers to Portugal and a group of children and adults on a Mediterranean cruise ship (ICAIR, 1984). Expatriates in endemic areas may also be at high risk. The incidence of giardiasis in expatriates in Bangladesh was found to more common than among new-comers

and children less than 10 years old (Rabbani and Islam, 1994). Epidemiological studies have also reported an increased risk of giardiasis among visitors to the Colorado mountains and hikers, backpackers, and campers in other areas who drink untreated or inadequately treated water from lakes and streams (ICAIR, 1984; Farthing, 1994).

A case-control study of 74 cases and 108 matched controls from July 1992 to May 1993 in Somerset and Avon, England, found that travel to developing countries was among several important risk factor for giardiasis (Gray et al., 1994). In a case-control study of 349 Washington State residents having *Giardia*-positive stools during July 1978 to March 1980 and 349 controls matched by age and gender, Frost et al. (1983) found that foreign travel to developing countries was associated with higher risk of acquiring infection for adults. A case-control study of 171 giardiasis patients in New Hampshire during January, 1977 to June, 1984 and 684 controls matched by age and gender also showed an increased risk of acquiring giardiasis associated with travel outside the United States (Chute et al., 1985, 1987). A case-control study of giardiasis in Vancouver (Mathias et al., 1992) found that travel abroad and travel within British Columbia were important risk factors. Isaac-Renton and Philon (1992) found higher risks of giardiasis among persons who traveled to rural areas of British Columbia and drank tap water or engaged in recreational water activities. A study of endemic giardiasis in Canterbury, New Zealand, travel abroad was a significant risk factor (Hunter, 1998).

7. Day-Care Centers

Outbreaks of giardiasis and a high prevalence of infection have been reported in settings where infants and young children in diapers are collectively cared for. Not only is the spread of *Giardia* likely within the care center but secondary transmission to family members is also likely. The occurrence and transmission of giardiasis in the child day care setting was recently reviewed by Thompson (1994). Thompson (1994) found prevalence rates of *Giardia* infection in Australia to range between 2% and 46% and to be highest among children 1-5 years of age who attended

preschool care. In three day-care centers in Atlanta, Georgia, the infection rate ranged from 29% to 54% compared to 2% among children not attending day-care centers; in two day-care facilities in metropolitan Toronto, Canada, infection rates of 17% and 39% were reported (ICAIR, 1984). The most susceptible were children 1 to 3 years old. Infections were reported to have been spread to as many as 23% of the children's household contacts (ICAIR, 1984). Secondary spread was found to be important within families having *Giardia*-positive children between 1- and 3-years-old; 10% of 47 family members studied were also *Giardia*-positive.

A two-year prospective study of diarrheal illness in children up to 36 months of age in 22 day care centers in Maricopa County, Arizona, identified 465 sporadic cases and 170 outbreak-associated cases of diarrhea (Bartlett et al., 1991). *Giardia* was significantly more common in toddlers than in infants and was found in 19% of asymptomatic child contacts of symptomatic infected children. In the second year, the study included children of the same age in 30 day care homes and 102 households not using day care. The seasonal pattern of diarrhea, frequency of pathogen isolation, and relative frequency of individual pathogens were similar in each setting. *G. lamblia* and rotavirus were the most commonly isolated enteropathogens.

In 1989 and 1990, a survey of stool specimens from 292 diapered children attending 17 randomly selected day-care centers in Fulton County, Georgia, found that 21 (7%) children in 7 centers were infected with *Giardia* (Addiss et al., 1991). Infected children ranged in age from 3 to 30 months, and only 57% of Giardia-positive children had symptoms. In 1986 the prevalence of infection in these same centers had been higher, 11% (Addiss et al., 1991). Both prevalence rates, however, are lower than the 16% to 38% infection reported in other studies for children attending non-outbreak centers (Addiss et al., 1991). The percentage of day-care centers with one or more infected children (41%) was also less than the 66% to 85% that had been reported in other studies (Addiss et al., 1991). In addition to person to person spread, a possible role was suggested for fomites in the transmission of *Giardia* in day-care centers. Laboratory studies found that *Giardia* cysts survive for less than 24 hours on dry environmental surfaces, and unless

surfaces are continuously being contaminated, a child's risk of exposure from to *Giardia* from fomites is limited to the day of contamination (Addiss et al., 1991). Another survey of 80 of 231 children 2 to 3 years of age in six commercial day care centers found that 13 (16%) were infected with *Giardia* and that five family members from four different families were positive (Cody et al., 1994). Only seven of the infected children (54%) had diarrhea. No care-givers were found to be *Giardia*-positive. Oretega and Adam (1997) reported that no seasonal pattern has been observed for *Giardia* infection in day-care situations; however, Rodriguez-Hernandez et al. (1996) observed a higher frequency of giardiasis in the autumn season in a study of eight day care centers in Salamanca, Spain. *G. intestinalis* was identified in 25% of the children studied.

An epidemiological study of endemic cases of giardiasis not associated with outbreaks reported from 1983 to 1986 in Vermont found that person to person transmission in child-care facilities was important in the transmission of non-outbreak cases of giardiasis.(Birkhead and Vogt, 1989). Children aged one to four years had the highest incidence rate for symptomatic infection of any age group, and child-care attendees had an incidence rate 50% greater than non-attendees (300.0/100,000 versus 194.7/100,000). Harter et al. (1982) found no differences in prevalence of infection between children who normally attended day-care centers and those who did not and found no correlation between the socioeconomic status of the families and the presence of *Giardia*. An important risk factor identified by Harter et al. (1982) was having two or more siblings between the ages of 3- and 10-years-old.

Epidemiological investigation of a foodborne giardiasis outbreak in a Minnesota nursing home also suggested person to person transmission, as illness in residents was associated with physical contact with children at the day care facility through an adopted grandparent program (White et al., 1989). Steketee et al. (1989) described three outbreaks of giardiasis that occurred over a 19-month period in a Wisconsin facility that cared for a daily average of 115 children aged 1 month to 6 years. Estimated attack rates in the three outbreaks were: 47%, 17%, 37% for children; 35%, 13%, 9% for staff; and 18%, 9%, 5% for household contacts. Infections recurred

in outbreak proportions even though a variety of control measures were instituted, including pharmacological treatment with a cure rate of >90%, better case identification, follow-up testing of stools, and improved personal and environmental hygiene practices. Bartlett et al.(1991) found *Giardia* infection in almost 11% of 676l new infants and toddlers tested for admission to 31 day care centers. A prospective randomized trial comparing three strategies for control of *Giardia* in infant-toddler day care centers found that more strict intervention (exclusion and treatment of both symptomatic and asymptomatic infected children) did not result in better control of infections; an initial *Giardia* prevalence of 18-22% in the three intervention groups was reduced to 7-8% in each group at 6 months intervention (Bartlett et al., 1991).

Steketee et al. (1989) found that attack rates were highest among the ambulatory children in diapers, children who attended the center for 40 or more hours per week, and children who had been infected in the respective previous outbreak. This latter finding suggests that giardiasis infection may not provide immunity for subsequent re-infections in this age group. Acquired immunity to giardiasis in adult populations has been suggested by epidemiologic studies, and there is experimental evidence of acquired immunity in mice; however, it is possible that immunity may not be established in young children or the immune response may be reduced by early drug therapy (Steketee et al., 1989).

8. Sensitive Populations

Immunodeficiency with varying degrees of hypogammaglobulinemia or agammaglobulinema predisposes to the acquisition of giardiasis and is the most commonly reported form of immunodeficiency associated with chronic giardiasis (Farthing, 1996). Giardiasis is more prevalent in homosexual men both with and without human immunodeficiency virus (HIV) infection (Farthing, 1996). In a selected New York City population examined for parasitological diseases by the same laboratory using the same procedures, 18.3% of 126 homosexual males were found to be cyst-positive for *Giardia*

compared to a 2.1% positivity among the other 5,885 patients (ICAIR, 1984). Giardiasis can be transmitted by some sexual activities, particularly among male homosexuals who practice oral-anal sex (ICAIR, 1984; Turner, 1985; Farthing, 1996). Chronic giardiasis is not a major clinical problem in persons with HIV infection or acquired immunodeficiency syndrome (AIDS) patients (Farthing, 1996). It is not clear why the intracellular protozoa (*Cryptosporidium parvum*, microsporidia, and *Cyclospora*) produce severe chronic diarrhea in AIDS patients but the effect of giardiasis is relatively mild (Farthing, 1996).

H. Environmental Factors Affecting the Survival of *Giardia* Cysts

1. Effects of Water Temperature on *Giardia* Cyst Survival

Temperature is a significant factor in the survival of *Giardia* cysts. Information reviewed in the *Giardia* criteria document (ICAIR, 1984) indicated that cysts suspended in tap water could survive more than two months when held at temperatures of 8°C, about 26 days at 21°C, and about 6 days at 37°C. Fewer than 1% of the cysts survived freezing at -13°C for 14 days and raising the temperature of cyst suspensions to boiling immediately inactivated the cysts. These data had been developed using *G. lamblia* cysts and excystation as the indicator of viability.

Using excystation with *G. muris* cysts, Schaefer et al. (1984) determined that the cysts were inactivated after freezing in distilled water at -20°C and then thawed. The length of time the cysts remained frozen was not specified. These investigators also found that the thermal death point (the lowest temperature at which the organisms are inactivated in 10 minutes) for *G. muris* cysts was 54°C. Tests (deRegnier et al., 1989) have also been conducted tests on the viability of *G. muris* cysts in fecal pellets when stored in different types of waters (distilled, lake, river and tap). The cyst viability measurements were based on exclusion of a fluorogenic dye propidium iodide (PI), mouse infectivity, and cyst morphology by Nomarski microscopy. In tests with cysts stored in distilled water in the refrigerator (5° to 7°C), viability by PI was 83% to

90% at 7 days, 13% to 25% at 28 days, and less than 1% at 56 days. Viability by mouse infectivity testing was 100% at 7 days, 17% to 100% at 28 days, and 0% at 56 days. In storage tests in both natural lake and river waters, these investigators reported that only decreased water temperature was correlated with survival. They observed longer viability at lower temperatures with cysts stored at <10°C remaining viable for 2 to 3 months. Other water quality parameters that were tested and for which no correlation was found were: pH, dissolved oxygen, turbidity, color, hardness, ammonia, nitrate and phosphorous.

Cysts were also exposed to tap water (deRegnier et al., 1989). Cysts in fecal pellets were placed in glass vials containing Minneapolis city tap water, and the vials were suspended in flowing tap water (20° to 28°C). The viability at 7 days was <2%, based on PI dye exclusion, and 0-17% viable, based on mouse infectivity testing. At 14 days, no viable cysts were detected by either PI or mouse infectivity. The authors were surprised by the loss of viability when the cysts were exposed to tap water for as little as 3 days as compared to survival of cysts in unprocessed river water. They indicated that the factors responsible for the cysticidal effect had not been determined but that the effect was most likely due to residual chloramine.

Although the viability was not determined, Erlandsen et al. (1990d) studied the effects of freeze-thaw cycles on the recovery of *G. muris* and *G. lamblia* cysts. *G. muris* cysts at levels ranging from 10^4 to 10^6/mL were suspended in an unspecified medium and frozen at -16°C and thawed at room temperature (approximately 20°C) through either one or three cycles. After one freeze-thaw cycle there was no detectable loss of cysts in preparations with high levels of cysts. However, there was about a 40% to 60% loss of cysts from preparations with low levels of cysts. After three freeze-thaw cycles, the loss in the high level preparations was about 22% to 27% and in the low level preparations, about 70% to 80%. These investigators also indicated that while the cysts were detectable with IFA staining, only 10% or less were recognizable using bright field microscopy. *G. lamblia* cysts were easily detected with IFA but immunostaining was variable and this was attributed to freeze-thaw damage.

2. Other Factors that Affect *Giardia* Cyst Survival

Since soil surface disposal of mixed human and animal wastes is a possibility, Deng and Cliver (1992) studied the survival of *G. lamblia* cysts in mixed human wastes (septic tank effluent or STE) and swine wastes (swine manure slurry or SMS). They used PI exclusion as an indicator of cyst viability. Under field conditions, the degradation rates of the cysts suspended in either buffer or STE were similar as determined by D values (the time in days for a 90% reduction in the number of cysts). The D values for viable cysts in buffer and STE were 16.9 and 17.6, respectively. However, mixing STE and SMS greatly increased the rate of degradation producing a viable cyst D value of 3.7. The temperature significantly influenced the rate of degradation in mixed wastes. The D values for viable cysts at 5, 15 and 25°C were 129.9, 26.2 and 4.1, respectively. The authors reported that they did not determine the mode of degradation but they postulated that bacteria may have been involved. They indicated that STE and SMS are both rich in bacteria and that electron micrographs showed bacteria adhered to the cyst walls.

Land application of municipal wastewater treatment plant sludges is a disposal option that prompted Van Praagh et al. (1993) to study the inactivation of *Giardia muris* cysts in anaerobic digester sludge. Cysts were seeded into sludge in the laboratory, anaerobic conditions were simulated in air-tight containers, and samples were taken at various exposure intervals. Cyst inactivation was determined based on original and final cyst levels, and the fraction of the original cysts which exhibited excystation. There were 99.9% cyst inactivations at 15.1 days, 20.5 hours, and 10.7 minutes with exposures at 21.5°, 37°, and 50°C, respectively. Casson et al. (1990) sampled activate sludge and trickling filter effluents at a Maryland wastewater plant finding a geometric mean of 4 cysts/L and 11 cysts/L, respectively. The plant influent contained a geometric mean of 137 cysts/L. Cysts were concentrated in suspended solids and in the sludge.

Johnson et al. (1997) investigated the survival of *G. muris* cysts and other enteric pathogens in marine waters in Hawaii. The viability of the cysts, as determined by excystation,

was reduced by 99.9% in only 3 hours when the cysts were suspended in marine waters and exposed to sunlight. However, when the cysts were kept in the dark, 77 hours were required to obtain a 99.9% reduction. In two marine waters with different salinities (28 and 35 mg/L), cysts survived longer in the lower salinity water. However, since different waters were used, the potential effect of factors other than salinity cannot be ruled out.

Rodgers et al. (1998) have isolated a bacterium from a Kentucky stream that can kill *G. lamblia* cysts. The bacterium has been characterized as a Gram negative, aerobic rod and it produces a yellow pigment not of the flexirubin type. The organism, designated Sun4, produces a spreading colony morphology on low nutrient agar although true gliding motility has not been observed. Using ribosomal RNA sequencing and phylogenetic analysis, the organism has been identified as a *Flavobacterium* most closely related to *F. columnare.* Static cultures, as opposed to shaken cultures, are more effective in killing cysts and calcium must be present in order for the bacterium to grow and to kill cysts. The intact bacterial cells must be present for cyst inactivation to occur as evidenced by the ineffectiveness of cell-free extracts. The authors suggest that Sun4 or other bacteria might be used as biological control agents for *Giardia* cysts in drinking water.

I. Summary

1. Occurence

Interpretation of the occurrence data for *Giardia* in water and other environmental samples is dependent upon methods used to detect and quantify the cysts. Methods used to date generally provide little or no information on viability/infectivity or species identification of organisms to assist in assessing the epidemiological significance of positive findings of cysts in environmental samples. Quantitative data may not be reliable due to low efficiency and precision of methods.

Giardia cysts are distributed worldwide in surface waters of all qualities. Cysts have been found in surface waters from the Arctic to the tropics. All municipal wastewaters and surface waters likely always contain *Giardia* cysts at some level. Whether or not they are detected is dependent upon the methods used. Cyst densities that have been reported generally are on the order of 10^{3-4}/L in raw sewage; 10^{1-2}/L in secondary treated wastewaters, and 10^{0}/L or less in surface waters. Generally, there is no correlation of cyst densities in water with bacterial indicator organisms. Cysts occur in surface waters throughout all months of the year. Occasionally, seasonal variations are reported but these may be site or region specific. When they are reported in North America, the levels are generally higher in the late summer, fall and early winter.

Longitudinal studies using high frequency sampling indicate spikes in cyst levels that might be missed by monitoring programs using low frequency sampling schedules. Cyst levels are generally higher in rivers or streams impacted by agricultural (e.g., cattle or dairy farming) or residential (e.g., sewage outfall) activities.

Levels of *Giardia* are usually reported to be somewhat lower than *Cryptosporidium* densities in U.S. waters. In other countries, e.g., Canada, widespread surveys have produced the opposite results.

National, regional, state or local surveys for occurrence of cysts in water may not be predictive of what will be found in a specific watershed. Sources of contamination and factors affecting transport and survival of cysts need to be determined for each watershed. Contamination levels of sources may fluctuate significantly due to poorly defined factors including weather events, agricultural practices and treatment plant (wastewater and drinking water) infrastructure and practices. The first-flush waters from storm events have been found to significantly affect source water cyst occurrence.

No published reports on the occurrence of *Giardia* in soil or air were found. One study reported the occurrence of cysts on stainless steel and Formica® surfaces in day care centers. A number of foodborne outbreaks have been reported but data are sparse to non-existent on quantitative levels of cysts in or on foods.

The viability and longevity of *Giardia* cysts in the environment is significantly affected by temperature–as the temperature increases, the survivability decreases. A small fraction of cysts can withstand a single freeze-thaw cycle. Cysts subjected to repeated freeze-thaws as might occur in the environment are likely inactivated but still will be detected with present methods.

Cyst inactivation in municipal wastewater treatment plant sludges is temperature-dependent. There is a factor or factors in swine manure slurry that results in more rapid degradation of cysts under field conditions. A bacterium has been isolated from a fresh water stream that is capable of killing *Giardia* cysts.

2. Prevalence, Outbreaks, and Endemic Risks

Giardiasis affects all age groups. High risk groups for giardiasis include infants and young children, travelers to developing countries, the immunocompromised, homosexuals who practice oral-anal intercourse, and persons who consume untreated water from lakes, streams, and shallow wells. Populations in communities with unfiltered surface water or groundwater that has been contaminated by surface water or sewage are also at high risk of infection. Waterborne outbreaks are more common in the United States and Canada than Europe, and this may be due to the larger number of unfiltered surface water systems in North America. Waterborne outbreaks of giardiasis can occur when disinfection is interrupted, disinfection levels are inadequate, disinfection contact time is low, or turbidity levels are increased, especially in areas where water temperatures are low. In low water temperatures, water disinfection may be less

effective and *Giardia* cysts survive for longer periods of time. In many waterborne outbreaks of giardiasis in the United States, neither the turbidity limit nor the coliform limit was exceeded.

Outbreaks of giardiasis in ground water systems emphasize the need to protect these sources from sewage and surface water contamination. Vulnerable ground water sources that cannot be protected should be considered to be at the same high risk of contamination as surface water sources.

Outbreaks of giardiasis that have occurred in filtered water supplies emphasize the need for proper chemical pretreatment and the importance of good design, installation, maintenance, and operation of treatment facilities. Ten percent of the waterborne outbreaks of giardiasis in the United States occurred as a result of contamination in the distribution system, and adequate precautions should be taken to protect treated water quality during storage and delivery.

Endemic risks of waterborne giardiasis are higher among persons who consume untreated water, and in the United States, Canada, and New Zealand, higher endemic risks have been identified among populations that consume unfiltered surface water.

Several small foodborne outbreaks of giardiasis have been associated with the contamination of ice used for beverages and foods by infected food service workers. Outbreaks have occurred in day-care populations, and the prevalence of *Giardia* infection is relatively high in these populations. However, risk factors for the introduction, spread, and persistence of *Giardia* in child day-care centers are not well understood.

IV. HEALTH EFFECTS IN ANIMALS

Giardia has been reported to infect virtually all vertebrate animals, including higher mammals (humans and other primates), domestic mammals (cats and dogs; cattle and sheep),

wildlife (beavers and muskrats), and other animals (birds, reptiles and amphibians). Calves may be jointly infected in a natural farm setting with both *Giardia* and *Cryptosporidium* (Xiao et al., 1993).

H. Symptomatology

Several reports, supported by light micrographs, have appeared in the literature, suggesting that *Giardia* trophozoites penetrate the mucosal cells of the intestine, as well as various extra intestinal tissues in rodents (ICAIR, 1984). The signs of symptomatic giardiasis in animals include diarrhea, steatorrhea (i.e., excessive discharge of fat in the feces), anorexia, weight loss, and vomiting (ICAIR, 1984), and in general, are similar to symptoms observed in humans. See Chapter V for a discussion of human health effects.

The signs and clinical picture of symptomatic giardiasis in primates observed at the Kansas City Zoo were similar to those seen in their human attendants, who also contracted the disease (ICAIR, 1984). Although no fatalities were reported, all patients suffered from loose stools, diarrhea, and vomiting. Since all infected apes and monkeys received chemotherapy, it is not known whether the giardiasis in these animals was a self-limiting disease.

In a study of calves naturally infected with *Giardia*, all infected animals were noted to have intermittent diarrhea, and mucus was seen in many fecal samples (Ruest et al., 1997). In mice inoculated with *Giardia* cysts, impaired weight gain and diarrhea were observed (ICAIR, 1984). The infection was observed to clear spontaneously, with most animals appearing to be free of giardiasis symptoms within 21 to 28 days. In parakeets, giardiasis has been associated with diarrhea, decreased intake of food and water, debility, and high mortality ranging from 20 to 50% (ICAIR, 1984). Diarrhea, anorexia, and occasionally, cessation of fecal elimination were observed in chinchillas infected with *Giardia* (ICAIR, 1984). Not all of the chinchillas infected became symptomatic, but of the four that had symptoms, three died.

Household pets have also been observed to show signs of *Giardia* infection including weight loss, mucoid and soft stools, and the presence of split and unsplit fats in the feces (ICAIR, 1984). Dogs less than one-year-old appear to be more likely to have symptomatic giardiasis than older dogs (ICAIR, 1984). Signs in these canines include diarrhea with mucus and fats, listlessness, and anorexia. In puppies, severe giardiasis may result in complications ranging from growth retardation to death (ICAIR, 1984).

B. Therapy

At present, no drugs are approved for treating giardiasis in animals. The benzimidazoles, albendazole and fenbendazole, have been shown to clear *Giardia* cysts from the feces of infected dogs (Barr et al., 1993; Zajac et al., 1998). Because albendazole is suspected of being teratogenic, it should not be given to pregnant animals.

Fenbendazole can be used safely to treat giardiasis in dogs, including pregnant and lactating animals (Barr et al., 1994). Other drugs, including quinacrine hydrochloride and metronidazole, have been used with varying degrees of success to treat giardiasis in dogs (Zimmer and Burrington, 1986). Olson et al. (1997c) found that immunization of puppies provided protection against giardiasis. Twenty puppies were vaccinated with a trophozoite-derived *Giardia* vaccine and boosted on day 21; 10 control puppies received only saline. Both groups were experimentally infected on day 35 with 1 x 10^6 *Giardia duodenalis* trophozoites by intraduodenal injection.

Giardiasis in cats can be treated with albendazole, but it should not be administered to pregnant animals. Metronidazole or furazolidone may also be used for *Giardia* infections in cats (Kirkpatrick, 1986; Patton 1998).

McAllister et al. (1996) found that the number of *Giardia* cysts shed in feces of growing lambs was not affected by salinomycin in their diet but did decline with time. Although a beneficial effect of 10 mg/L salinomycin on lamb performance was seen, the development of natural resistance made it difficult to attribute this response to the control of giardiasis.

Giardia cyst excretion in naturally infected calves was shown to be reduced or eliminated after treatment with albendazole or fenbendazole (Xiao et al., 1996; O'Handley et al., 1997). Calves may be also treated with quinacrine hydrochloride, ipronidazole or dimetridazole. *Giardia* infection in horses can be cured with metronidazole. Finally, giardiasis in large animals and birds may also be treated orally with fenbendazole (Patton, 1998).

C. Epidemiological Data

Epidemiological data (Erlandsen et al., 1988a, b) show that *Giardia* infection in animals:

(a) is spread via the fecal-oral route;

(b) occurs worldwide, in most animal species;

(c) is more often than not asymptomatic;

(d) is primarily a disease of the young (suggesting a role for immunity in these infections);

(e) is much more likely to spread within a host species than from one host species to another.

Some information is available about the prevalence of *Giardia* in lower animals. In general, the prevalence data are based on the examination of animals selected with no regard for their symptoms. The prevalence of *Giardia* infection in beaver was found to be 7% to 16% in different parts of the United States, and in muskrats the prevalence was greater than 95% (Erlandsen et al., 1990c). *Giardia* infection was found in 153 (77%) of 200 dogs and 9 (3%) of 300 cats tested in Minnesota (Bemrick 1961). Similar prevalences were reported in Spain

(Lopez-Brea, 1982) and Japan (Miyamoto and Kutsume, 1978; Asama et al., 1991; Arashima et al., 1990). Kirkpatrick (1986) reported the prevalence of *Giardia* infection in cats to range from 1 to 11 percent in the United States. In Washington, Pacha et al. (1987) found that 469 (65%) of 722 of the fecal samples collected from a variety of animals including voles, mice and shrews were positive for *Giardia*.

The results of recent studies underscore the fact that *Giardia* is a common protozoan parasite of farm animals (including cattle, sheep, pigs, and horses) and occurs with greater prevalence in young, than in adult, animals (Buret et al., 1990; Olson et al., 1997a). Buret et al. (1990) found the prevalence of *Giardia* infection was 18% in sheep and 10% in cattle and was significantly higher in lambs and calves (36% and 28%, respectively). Olson et al. (1997a) also found *Giardia* to be a prevalent infection in farm livestock; 104 cattle, 89 sheep, 236 pigs and 35 horses were sampled from 15 different locations in Canada. *Giardia* were present in cattle and sheep in all six sites sampled with a prevalence of 29% and 38%, respectively; the prevalence was greater in calves and lambs. All horse sampling locations were positive for *Giardia* with 20% of animals infected. *Giardia* was identified in four of six hog operations with a prevalence of 9%.

Bettiol et al. (1997) found *Giardia* in 21% of 295 Tasmanian native marsupials screened over a three-year period. After isolating immunologically-confirmed human-infective *Giardia* from two Australian marsupials, the northern brown bandicoot (*Isoodon macrouris*) and the red-necked pademelon (*Thylogale thetis*), Buckley et al. (1997) suggested that the potential exists for the waterborne transmission of human-infective *Giardia* in pristine watersheds of Australia even though humans and domestic livestock are excluded. Buckley also noted the isolation of *Giardia* from Australian bushtail possums.

Olson et al. (1997b) identified *Giardia* in the intestinal contents of three of 15 ringed seals (*Phoca hispida*) slaughtered by Inuit hunters in the western arctic region of Canada.

Giardia has also been identified in llamas (Rings and Rings, 1996) and a captive population of marmosets (Kalishman et al., 1996). Finally, wading birds (blue herons, egrets, green herons and black crowned night herons) have been reported to have *Giardia* prevalence rates greater than 90% (Erlandsen, 1994; Erlandsen et al., 1990b). McRoberts et al. (1996) describe the morphological and molecular characteristics of *Giardia* isolated from a straw-necked ibis in Australia.

D. Summary

In general, the symptoms seen in lower animals resemble those seen in humans. Many, if not most, animals with *Giardia* infection exhibit no symptoms. These animals do, however, serve as sources of infection for other animals. In those animal species (e.g., cats and dogs) whose *Giardia* infections have been studied in detail, the epidemiology is similar to humans. That is, *Giardia* infections may occur in animals of any age, but they are more likely to occur, and to be symptomatic, in young animals. Symptomatic infections in animals that require therapy usually respond to the same agents, with the same caveats, used in treating human infections. Mortality is rare in humans but appears to be significant in some animals, e.g., chinchillas and budgerigars.

V. HEALTH EFFECTS IN HUMANS

A. Symptoms and Clinical Features

Giardia infection is often asymptomatic. Asymptomatic cases may represent as many as 50% to 75% of infected persons (Mintz et al., 1993). In a study at the Swiss Tropical Institute, only 27% of 158 patients who had *Giardia* cysts in their feces exhibited symptoms (ICAIR, 1984). Infection may also be associated with a variety of intestinal symptoms including chronic diarrhea, steatorrhea, abdominal cramps, bloating, flatulence, pale greasy and malodorous stools, and weight loss (ICAIR, 1984; Benenson, 1995). Nausea or vomiting may also occur (Hopkins and Juranek, 1991). Fever is occasionally present at the beginning of the infection (Ortega and Adams, 1997). Blood is not present in stools unless it is due to anal irritation from the diarrhea (Wolfe, 1990). Malabsorption of fats or of fat-soluble vitamins may also occur (Benenson, 1995). For example, subnormal fractional absorptions of folate and vitamin B12 were found in one-sixth and one-third, respectively, of 29 Swedish children, age 8 months to 13.5 years, with chronic giardiasis (Casterline et al., 1997).

Giardia trophozoites principally infect the small intestine. There is usually no extra-intestinal invasion, but reactive arthritis may occur (Shaw and Stevens, 1987). In severe giardiasis, duodenum and jejunal mucosal cells may be damaged (Benenson, 1995). Cases of severe, reversible impairment in pancreatic function have also been reported (Carroccio et al., 1997; Nakano et al., 1995). Uveitis and urticaria have been observed in several patients with giardiasis but may have been coincidental (ICAIR, 1984). Inflamation of the synovial membranes of major joints has also been seen in children with giardiasis, but following anti-giardial chemotherapy, intestinal and synovial symptoms were abated (ICAIR, 1984).

Infection is frequently self-limited, but persons with AIDS may have more serious and prolonged infection (Benenson, 1995). Immunocompromised persons, especially those with acquired immune deficiency syndrome (AIDS) and achlorhydria may be more susceptible to

symptomatic infection (ICAIR, 1984). Immunodeficiency with varying degrees of hypogammaglobulinemia or agammaglobulinema is the most commonly reported form of immunodeficiency associated with chronic giardiasis (Farthing, 1996). Giardiasis is one of the few potentially treatable causes of diarrhea in persons with AIDS, and although *Giardia* infection is not as prevalent as other pathogens in AIDS patients, it is important that the infection be accurately diagnosed (Hewan-Lowe, 1997). Co-infection with *Giardia lamblia* and *Enterocytozoon bieneusi* was detected by endoscopically obtained small intestine biopsies from a patient with AIDS and chronic diarrhea who had repeated negative stool examinations for ova and parasites (Hewan-Lowe, 1997).

Deaths due to giardiasis are rare; CDC reported that giardiasis had caused only four deaths in the United States in 1982 (ICAIR, 1984). An estimated 4,600 persons were hospitalized with giardiasis annually in the United States from 1979 to 1988 with a median length of hospital stay of 4 days (Lengerich et al., 1994). Volume depletion or dehydration was the most frequently listed co-diagnosis on admission, and almost 19% of the children younger than 5 years of age who had severe giardiasis also were diagnosed with failure to thrive (Lengerich et al., 1994). In Scotland, the median length of stay in the hospital for giardiasis was significantly longer for persons older than 70 (11 days compared to 3 days) than for other age groups (Robertson, 1996). Dehydration did not occur as frequently with giardiasis in Scotland, either because of *Giardia* strain differences or because rehydration treatments are more widely self-administered in Scotland. Some 11% of the children who were hospitalized for giardiasis in Scotland were also found lacking in expected normal physiological development (Robertson, 1996).

The duration of acute clinical illness may vary greatly. In some patients, symptoms last for only 3 or 4 days, while in others the symptoms last for months. Generally, patients commonly resolve their infections spontaneously, and acute disease lasts from 1 to 4 weeks (ICAIR, 1984). In some patients, the acute stage may last for months (Wolfe, 1990). In

untreated patients, the median duration of illness is six weeks with symptoms lasting less than one week (Adam, 1991).

The period of communicability lasts for the entire duration of infection, and during infection, the shedding of cysts can be intermittent (Benenson, 1995). Although persons with asymptomatic *Giardia* infection are not likely to seek medical treatment and be diagnosed, they can serve as unidentified carriers of infection. Carrier infections may last for months or years (ICAIR, 1984). Asymptomatic *Giardia* infection for children may be epidemiologically significant (ICAIR, 1984). Infected children in day-care centers are frequently asymptomatic but can transmit the infection to other children, care givers, and family members (Ortega and Adam, 1997). In a longitudinal study, almost 15% of 82 children in a day-care center excreted cysts for a mean of six months (Turner, 1985).

When giardiasis is suspected, it is advisable to confirm that *Giardia* is the cause of the illness. For patients with chronic diarrhea, upper abdominal cramps, and "frothy" stools, the examination of up to three concentrated stool specimens are recommended (Donwitz et al., 1995; Conboy 1997). The collection of three stools has a sensitivity of 60% to 85% for detecting *Giardia* cysts (Donwitz et al., 1995). ELISA for determining *Giardia* antigen in stool (sensitivity 92%; specificity 98%) has largely supplanted intestinal biopsies, wet preparation, and the duodenal string test (Donwitz et al., 1995). Benenson (1995) reports that test kits are commercially available to detect the *Giardia* antigen in the stool. Howard et al. (1995) recently detected *Giardia* in biopsies of the colon and terminal ileum and suggested that physicians may wish to perform colonic or ileal biopsies when the clinical symptoms suggest giardiasis and the more routinely-performed duodenal aspirates or biopsies have been found to be negative. Diagnostic tests are more completely described in Chapter VII, Section B.

B. Epidemiology

Based on data from infected travelers to the U.S.S.R., the reported mean time period between infection and the onset of acute disease was 12 to 15 days, but the time ranged from 1 to 75 days (ICAIR, 1984). Ortega and Adams (1997) report the incubation period for symptomatic persons is one to two weeks but may vary from 1-45 days. Benenson (1995) reports that the incubation period is usually 3 to 25 days or longer, with a median of 7 to 10 days. In a prospective epidemiological study, Jokipii et al. (1985) found that the incubation period for giardiasis may typically be in the range of 12 to 19 days. In human volunteers inoculated with *G. lamblia* trophozoites by intestinal intubation, Nash et al. (1987) found that and that diarrhea or loose stools appeared within 7.25 (± 2.99) days of inoculation. In human volunteers fed human-source *Giardia* cysts, the incubation period of giardiasis (based on cyst detection in the feces) ranged from 9 to 22 days with a mean of 13.1 days (Rendtorff, 1954a, b; 1979).

In the United States and Scotland, more severe cases (i.e., hospitalized) of giardiasis seemed to occur primarily in children under the age of five (Lengerich et al, 1994; Robertson, 1996). Infants and young children may have increased susceptibility to giardiasis because of behavioral factors that increase exposure and immunological factors (Robertson, 1996). In Scotland, marked differences were found in the age distribution of hospitalized cases of cryptosporidiosis and giardiasis (Robertson, 1996). The median age for hospitalization of giardiasis was 30 years, whereas, the median age for cryptosporidiosis was 5 years, and the proportion of hospitalized cases in children under five was greater for cryptosporidiosis (49%) than giardiasis (28%). Robertson (1996) suggested this difference between severity of illness between these two protozoa may be because the development of protective immunity to *Giardia* infection is more prolonged than it is for *Cryptosporidium* infection. Development of protective immunity for *Giardia* infection has been considered to be relatively lengthy and does not necessarily result from a single infection (Farthing, 1994). The variation of antigenic profiles between *Giardia* isolates and its antigenic complexity also suggest there may also be more immunological sub-types of *Giardia*, and immunity may be specific for the particular sub-types

(Robertson, 1996; Rabbani and Islam, 1994). Immunity is discussed further in Section E of this chapter.

Giardiasis is transmitted via the fecal oral route of exposure, and both endemic and epidemic transmission are important. Although all age groups are affected, the highest incidence is in children (Benenson, 1995). Infants under 6 months of age who are breast-fed are not likely to be infected (Rabbani and Islam, 1994). It is a common cause of illness in travelers and often spread directly from person to person, especially among children or persons living in areas with poor sanitation and hygiene. Waterborne outbreaks have been reported, and some have resulted in a large number of cases of illness. Endemic waterborne giardiasis has been associated with drinking unfiltered surface water or shallow wells and swimming. Smaller outbreaks have resulted from contaminated food and person to person transmission in day-care centers. Oral-anal sexual activities among homosexuals has also been described as a risk factor (Turner, 1985).

Although people living in urban and rural areas may have different levels of risk of *Giardia* infection, both are at high risk of infection. In Zimbabwe, the annual incidence of the disease in urban children was 22%, compared to 12% for rural children (Rabbani and Islam, 1994). High population density in urban areas, overcrowding, poverty, and poor sanitation of the urban slum areas, especially in third world countries, contribute to the high rate of infection. Like other gastrointestinal infections, giardiasis is very common in populations living in poverty and with poor sanitation, and a high level of fecal contamination of the environment. Mason et al., (1986) indicates that even providing piped, clean drinking water to developing countries may not significantly reduce the incidence of giardiasis. Although contaminated drinking water is a likely source of exposure, the variety of other exposures including personal hygiene, food hygiene, and environmental factors may overwhelm the beneficial effect of clean drinking water. Further studies are required to understand the definite role of socio-environmental factors contributing to giardiasis.

C. Clinical Laboratory Findings and Therapeutic Management

1. Clinical Laboratory Findings

Clinical data suggest *Giardia* cysts are highly infective for humans. In a controlled, clinical study of male volunteers who were fed human-source *Giardia* cysts contained in gelatin capsules, a dosage of ten cysts was found to produce human infection, as determined by observing presence of *Giardia* in fecal smears (ICAIR, 1984). Eight dosage levels ranging from 1 cyst to 1 x 10^6 cysts per capsule were studied. Since cyst viability was not determined before being fed to volunteers, the failure to elicit infection in the five men treated with a dose of only one cyst may have been due to dosing with inactive cysts (ICAIR, 1984).

Nash et al. (1987) inoculated by intestinal intubation human volunteers with trophozoites of two distinct human isolates of *G. lamblia*, termed GS/M and Isr. Groups of five volunteers received 50,000 trophozoites of either isolate. None of the volunteers receiving Isr became infected, but all of the group inoculated with GS/M became infected. Of five volunteers inoculated and infected with GS/M, 3 became ill, with 2 showing diarrhea and other signs typical of giardiasis. These data suggest there are strain variations for *G. lamblia* and confirm in a controlled clinical setting that infected persons can exhibit a range of symptoms in addition to being asymptomatic.

Although one species of *Giardia* is believed to infect humans, the epidemiology of giardiasis is complicated by apparent genetic heterogeneity in this species (Thompson et al., 1993; Erlandsen, 1994; Nash et al., 1987). Differences in virulence, pathogenicity, infectivity, growth, drug sensitivity, and antigenicity have been reported (Thompson et al., 1996). Genetic diversity in the species of *Giardia* believed to infect humans is extensive with some clones widely distributed and others localized, especially in areas where endemic infection is high (Thompson et al., 1996). In these endemic areas where extensive heterogeneity of *Giardia*

exists, mixed infections with more than one genotype may occur and interference with clonal competition may have an important influence on the genetic variation (Thompson et al., 1996). Upcroft et al. (1995) conducted a long term study of the biology and genetics of *Giardia* after establishing in vitro and in vivo *Giardia* cultures in mice from 1829 duodenal aspirates collected over an eleven year period from children who were being treated for diarrhea and failure to thrive and in whom stool examinations were negative. Based on biochemical characteristics of electrophoretic karyotype, RFLP analysis and rDNA hybridization studies, at least two distinct varieties, or demes, of *Giardia* were found to have infected the population of the South East Queensland area of Australia. From 1983 to 1991 only one variety was documented, but in 1991 a new variety was seen with a predominance of this deme beginning in 1993. Since all of the stocks were derived from children with similar chronic symptoms it appears that at least two demes of *Giardia* were pathogenic in the South East Queensland area of Australia.

Thompson et al. (1996) has suggested that the regular suboptimal application of chemotherapeutic regimes may be a contributing factor to the persistence of genetic heterogeneity and that this, combined with the variable sensitivity of *Giardia* to these drugs, may inhibit competitive interactions between clones of *Giardia*. Competitive interactions studied in vitro found that competition occurred between genetically distinct isolates of *G. duodenalis* and that exposure to metronidazole has differential effects on growth of the clones; however, although these are necessary conditions, they are not sufficient to support suggestions that genetic heterogeneity is due to regular suboptimal drug treatments (Thompson et al., 1996)

2. Therapeutic Treatment and Management

As with all diarrheas, fluid replacement is an important aspect of treatment. Anti-giardial drugs are also important in the management of the giardiasis. Chemotherapeutic agents used for treatment of giardiasis are listed in Table V-1 (Benenson, 1995; Rabbani and Islam, 1994; Bulut et al., 1996; Freeman et al., 1997; Farthing, 1996; Adam, 1991). The drugs may have different

effectiveness in their ability to clear *Giardia* and various doses and treatment periods are recommended for each drug. Important implications for the management of patients include problems of drug resistance and relapses that may occur (Benenson, 1995) and cross-resistance and treatment failures that can occur in the absence of resistance (Upcroft et al., 1990).

After inducing albendazole resistance in three different *Giardia* cultures following growth in successively increasing amounts of drug, Upcroft et al. (1996a) found that the cultures were resistant to concentrations of albendazole against *Giardia* in vitro at 4-10 times normally lethal concentrations. Albendazole-resistant *Giardia* were also cross-resistant to parbendazole (Upcroft et al., 1996a). Recovering a metronidazole-resistant strain of *Giardia* from a patient, Butcher et al. (1994) felt that an unsuccessful course metronidazole treatment for chronic giardiasis may have resulted in the selection of the resistant strain of the parasite. Quinacrine resistance was studied by Upcroft et al. (1996b). Quinacrine was found to be rapidly absorbed by sensitive trophozoites but actively excluded from resistant trophozoites. Upcroft et al. (1990) reviewed the evidence for drug resistance in giardiasis and suggested markers, such as DNA probes, be developed to provide methods for monitoring the spread of drug resistant *Giardia* in populations. Biochemical studies were also undertaken to determine the basis for this resistance (Upcroft et al., 1990). Metronidazole and furazolidone, which produce toxic radicals within the cell, have different biochemical mechanisms of action. At the molecular level, resistance to metronidazole is associated with DNA changes.

Metronidazole or tinidazole has been the drug of choice for giardiasis probably because the treatment period is short and compliance good (Farthing, 1996; Benenson, 1995). Quinacrine and furazolidone have also been commonly used (Freeman et al., 1997). Freeman et al. (1997) reports that metronidazole is not approved for therapy in *Giardia* infection in many countries, and Paget et al.(1989) has found *Giardia* cysts are resistant to metronidazole. Rabbani and Islam (1994) report that in the United States, metronidazole is not approved by the Food and Drug Administration (FDA) for treatment of giardiasis but is approved for amebiasis. Farthing (1996)

and Benenson (1995) note that tinidazole is also not approved by the FDA for giardiasis. Furazolidone is reportedly the only drug approved by the FDA for treatment of giardiasis (Ortega and Adam, 1997).

Table V-1 Chemotherapeutic Agents for Giardiasis

Drug	Duration of Treatment	Efficacy
Quinacrine	5-10 days	>90%
Metronidazole	2-14 days; single dose adults	>90%
Tinidazole	7 days; single dose adults & children	>95%
Furazolidone	7-10 days	>80%
Paromomycin	5-7 days	Low may be <50%
Albendazole	5 days	>90%
Ornidazole	single dose children	>90%

*Adapted from Benenson, 1995; Rabbani and Islam, 1994; Bulut et al., 1996; Freeman et al., 1997; Farthing, 1996; Adam, 1991.

Studies have evaluated the effectiveness of metronidazole and compared its effectiveness to other medications and in combination with quinacrine or d-propranolol, an adrenocepter antagonist that appears to inhibit the mobility and growth of *G. lamblia* (Freeman et al., 1997). A large single or repeated dose of 2.0 g or 0.25 g three times daily for 7 days is reported effective for adults; dosages of 5.0 to 7.5 mg/kg three times daily for seven days are effective for children (Freeman et al., 1997). Ellis et al. (1993) noted that *G. intestinalis* is often refractory to treatment with metronidazole. Some patients who fail to respond to a single dose of metronidazole have responded to a second therapy of 3 or 7 days duration (Freeman et al., 1997). A combined formulation of diloxanide furoate and metronidazole was successfully used to clear the parasite from all of their patients with giardiasis (Qureshi et al., 1997). Metronidazole

appears to have fewer side effects than furazolidone and quinacrine, but nausea, metallic taste, and headache may occur (Turner, 1985). Metronidazole and furazolidone have been found mutagenic and carcinogenic in animal experiments (Turner, 1985).

Tinidazole, a chemical relative of metronidazole, is reported to be equally or more effective than metronidazole (Freeman et al., 1997) and has fewer side-effects (Rabbani and Islam, 1994). A single dose of 2.0 g in adults (0.5 or 1.0 g in children) has been used with success (Rabbani and Islam, 1994; Nahmias et al., 1991). Mepacrine or quinacrine is still used in some parts of the world and has proved effective where metronidazole and tinidazole have been unsuccessful (Upcroft et al., 1995; Farthing, 1996); however, it is not available in a number of countries, and side-effects including the risk of psychoses have been reported (Boreham, 1991). Mepacrine may be a useful alternative to metronidazole in the United States, but its use often leads to yellow staining of the skin and conjunctivae (Rabbani and Islam, 1994). Albendazole, a benzimidazole derivative, has also been shown effective in vitro at concentrations 30 times lower than metronidazole (Rabbani and Islam, 1994). Misra et al. (1995) found that albendazole is as effective as metronidazole for treating giardiasis in children and does not produce the anorexia that is often seen with metronidazole treatment. It was found to be almost as effective as metronidazole in treating Bangladeshi children; 95% of those infected and treated with albendazole cleared the parasite compared to 97% clearance with metronidazole (Hall and Nahar, 1993). In addition, albendazole is less expensive and has fewer side-effects than metronidazole (Bulut et al., 1996). Dutta et al. (1994) found albendazole as effective as metronidazole in a study of 150 Indian children aged 2-10 years randomized to receive either a single dose of 400 mg of albendazole suspension, or 22.5 mg/kg/day of metronidazole in 3 doses for 5 consecutive days. Two days after completion of therapy, 97% of children in both treatment groups were *Giardia* free. Side effects were noted in 3 children in the albendazole group, but in 20 children in the metronidazole group. Pungpak et al. (1996) found that a seven day course of albendazole was effective with no serious side effects among 63 children and 15 adults in Thailand. Another

benzimidazole derivative, mebendazole has been found effective in treating giardiasis (Bulut et al., 1996; Adam, 1991).

In a randomized clinical trial of 48 infected children, Bulut et al. (1996) found that ornidazole was 100% effective in clearing *Giardia* compared to 93% effectiveness for metronidazole and less than 60% for mebendazole (Bulut et al., 1996). Ornidazole at 40 mg/kg was administered as a single dose with only minor side-effects in three children (urticaria, vertigo, nausea). Bassily et al. (1970) found that furazolidone treatment cleared the parasite in 80% of infected Egyptian adults; quinacrine and metronidazole were 100% and 95% effective, respectively. Furazolidone is widely used for children in the United States partially because it is available in pediatric suspension (Farthing, 1996). A *Giardia* clearance rate of 92% was reported in children treated with furazolidone (Craft et al., 1981). Furazolidone is well tolerated by most patients, but may cause a reaction if taken with alcohol and may induce hemolysis in patients with glucose-6-phosphate dehydrogenase deficiency (Rabbani and Islam, 1994). While furazolidone is an effective treatment for giardiasis, Elliott et al. (1998) warn of the potent cumulative inhibition of monoamine oxidase associated with the prolonged use of the drug with potential effects on blood pressure and mood disorder; interactions with antidepressant drugs and foods rich in tyramine should also be considered.

Turner (1985) recommended treatment of giardiasis be avoided in pregnancy unless symptoms cannot be controlled by conservative measures. Because it is poorly absorbed, paromomycin has been used to treat giardiasis in pregnant women in whom other drugs are contraindicated; the cure rate is variable and may be as low as 55% (Rabbani and Islam, 1994; Farthing, 1997).

Pearce et al. (1996) compared two published methods for assessing the in-vitro drug sensitivity of *Giardia duodenalis* to metronidazole or albendazole: inhibition of adherence and the ^{3}H-thymidine incorporation assay which radiometrically measures nucleic acid synthesis.

Because of the different modes of action of metronidazole and albendazole on *Giardia*, measuring the inhibition of adherence appears to be a more accurate indicator of trophozoite viability than measuring ^{3}H-thymidine incorporation. This finding emphasizes the importance of considering the biochemical mechanisms of action when evaluating drug sensitivity. Feeling that differences in enzyme characteristics between the parasite and host may lead to development of future chemotherapeutics for giardiasis, Swarbrick et al. (1997) sequenced the cytidine triphosphate synthetase genes from three diverse strains of *G. duodenalis* and found that they varied significantly from each other. Boreham et al. (1987) tested two stocks of *G. intestinalis* by the ^{3}H-thymidine uptake assay to determine their sensitivity to metronidazole, tinidazole, furazolidone and quinacrine. Each stock was composed of different populations of organisms and not homogeneous with respect to drug sensitivity, and this may, in part, account for treatment failures in giardiasis patients.

Pentamidine and 38 analogs of pentamidine were screened for in vitro activity against *G. lamblia* (Bell et al. 1991). All compounds were active against *G. lamblia* as measured by the ^{3}H-thymidine incorporation assay, but anti-giardial activity varied widely. The activity of the most potent anti-giardial agent, 1,3-di(4-amidino-2-methoxyphenoxy)propane compared favorably with furazolidone, metronidazole, quinacrine, and tinidazole. Gordts et al. (1985) evaluated the in vitro susceptibility 25 *Giardia* lamblia isolates to six commonly used antiprotozoal drugs; tinidazole was the most active drug. Metronidazole was equally active on all but one isolate, and furazolidone was the most active nonimidazole compound tested. More than 50% of the isolates were very susceptible to paromomycin, pyrimethamine, and chloroquine. Crouch et al. (1986) evaluated the in vitro sensitivity of *G. lamblia* to 23 chemotherapeutic agents; tinidazole, metronidazole, and furazolidone were found to have strong inhibitory effects on both growth and adherence, while mepacrine had a strong effect on growth only. Three drugs (mefloquine, doxycycline and rifampin) not previously used in giardiasis were also found to have significant in vitro activity and deserve consideration for clinical evaluation of efficacy. Farbey et al. (1995) examined 12 isolates of *G. duodenalis* from Caucasian hosts in the

Perth metropolitan area, 16 isolates from Aborigines in Western Australia, and a reference isolate P1C10 for their in vitro drug sensitivity to metronidazole, benzimidazole, and albendazole. Metronidazole showed the most resistance. In addition, it was found that isolates of *Giardia* obtained from Aboriginal hosts were significantly less sensitive to albendazole than those obtained from Caucasians. In vitro growth of *G. lamblia* was found to be highly sensitive to certain anthelmintic benzimidazoles (Edlind et al., 1990). Albendazole and mebendazole were 30- to 50-fold more active than metronidazole and 4- to 40-fold more active than quinacrine. Thiabendazole was less active. Since lack of intestinal absorption makes mebendazole an attractive anti-giardial agent, its in vitro activity was further characterized. Lethal activity was observed at a concentration fivefold lower than necessary for metronidazole. Attachment of cells to the culture tube was rapidly disrupted by mebendazole treatment, and the characteristic cell structure was grossly distorted. Azithromycin was found to produce significant growth inhibition of *G. intestinalis* in vitro (Crouch et al., 1990). Crouch et al. (1990) also found that the dyadic combinations of azithromycin-furazolidone, doxycycline-mefloquine, doxycycline-tinidazole and mefloquine-tinidazole were synergistic for inhibition of adherence of *G. intestinalis* in vitro, suggesting combinations may be worthy of consideration for chemotherapy of recalcitrant giardiasis. Meloni et al. (1990) compared the effects of albendazole against *G. duodenalis* in vitro with those of tinidazole and metronidazole, finding it to have superior potency. Trophozoite morphology, adherence and viability were markedly affected by albendazole, to a far greater extent than by either metronidazole or tinidazole.

Ponce-Macotela et al. (1994) evaluated in vitro anti-giardial activity of 14 species of plants in Mexico as anti-diarrheics and/or anti-parasitics. Trophozoites of *G. duodenalis* were incubated with plant extracts and their viability was ascertained. In vitro anti-giardial effects were seen in nine species; *Justicia spicigera* (muicle), *Lipia beriandieri* (oregano), and *Psidium guajava* (guava) were found to be superior to tinidazole.

D. Mechanism of Action

Giardia cysts survive in the environment, and when ingested pass through the stomach where the acid environment triggers excystation, which usually takes place in the duodenum. The trophozoites attach to the duodenal or proximal jejunal mucosa, probably via contraction of the ventral disk, and replicate by repeated binary division (Marshall et al., 1997). Attachment of trophozoites is essential for colonization of the small intestine and a prerequisite for *Giardia*-induced enterocyte damage (Katelaris et al., 1995). A predominant role for mechanical attachment via cytoskeletal mechanisms is suggested by in vitro studies in cultured human intestinal cells, but Katelaris et al. (1995) feel that lectin associated binding may also have a role in vivo. Abnormal structural changes may occur in the mucosa but are usually reversed after treatment (Hall, 1994).

In severe giardiasis, duodenum and jejunal mucosal cells may be damaged (Benenson, 1995). The severity of the diarrhea has been positively correlated with the severity of changes in the villus histology (ICAIR, 1984). The more severe the villus atrophy, the more severe the diarrhea. After anti-giardial chemotherapy, recovery of the villus architecture occurred as diarrhea disappeared. Structural changes often involve a flattening of the mucosal surface and a change in the ratio of the length of crypts and villi; this could be responsible for malabsorption (Hall, 1994). The relationship between clinical disease and structural change in the mucosa is not always consistent, but the presence of inflammatory cells in the lamina propria seems to be common (Hall, 1994). A case of giardiasis was reported in a female college student whose symptoms persisted for 5 months and no villus atrophy was noted, but marked round cell infiltration occurred in the lamina propria (ICAIR, 1984). Because coeliac disease, a result of an immune response to gluten, leads to an enteropathy and inflammation similar to that seen in giardiasis, Hall (1994) suggests that diarrhea and other symptoms of giardiasis result from an inflammatory response to infection. It has been proposed that a toxic excretory or secretory product could be responsible for diarrhea, but the presence of a toxin has not been found nor is there evidence showing that *Giardia* is toxigenic (Hall, 1994; ICAIR, 1984). Other proposed

mechanisms for diarrhea include disruption of the brush border or immunopathologic processes (Ortega and Adam, 1997). Studies of Mongolian gerbils infected orogastrically with trophozoites suggest that an altered gastrointestinal transit and smooth muscle contractility may be involved in the pathophysiology of giardiasis (Deselliers et al., 1997).

Trophozoites do not normally penetrate the intestinal epithelium in humans, but evidence of mucosal invasion has been seen in patients who had diarrhea and large numbers of trophozoites in the lumen; similar invasion has not been seen in asymptomatic persons (Ferguson et al., 1990). An ultrastructural study of mouse infection found mucosal invasion only in areas where necrosis or mechanical trauma was present, and reports of mucosal invasion in humans are suspect if specimens were obtained by forceps biopsy or peroral suction (Ferguson et al., 1990). Chavez et al. (1995) found that all strains of *G. lamblia* recovered from children with symptomatic and asymptomatic giardiasis produced focal regions of microvilli depletion in vitro (MDCK epithelial cells), but none of the isolate strains were invasive.

Several mechanisms have been suggested to account for nutrient absorption abnormalities, including mechanical blockage of mucosal surfaces, functional mucosal changes brought on by invading trophozoites, mucosal damage from inflammation even in the absence of actual invasion, associated bacterial overgrowth, deconjugation of bile acids, and interference with lipolysis (Hall, 1994; ICAIR, 1984). However, experimental evidence for only the latter mechanism was found in the pre-1984 literature and the results were equivocal (ICAIR, 1984). Laboratory experiments suggested *Giardia* may interfere with the active transport of glucose and glycine, but not with the passive transport of potassium, and that the defect in the active transport mechanism might be due to structural damage of the substrate carrier or an alteration in cell maturation due to *Giardia* (ICAIR, 1984).

As noted in Sections A and C of this chapter, clinical effects range from the asymptomatic carrier state to severe malabsorption syndrome. Similarly, histopathological

changes in the affected mucosa may be minimal, or there may be significant enteropathy with enterocyte damage, villus atrophy, and crypt hyperplasia (Ferguson et al., 1990). Reasons for this variation in host susceptibility are poorly understood (Ferguson et al., 1990). Hall (1994) concluded that the mechanism by which *Giardia* causes disease and effects nutrition is unclear.

E. Immunity

Data supporting the role of acquired immunity to giardiasis comes from studies of both humans and animals. Results of epidemiological studies, studies of immunosuppressed human populations, animal experiments where artificial immunosuppression was induced, and studies of the immune and physiological reactions to *Giardia* infection of both humans and animals are all consistent with the development of protective immunity to illness from prior *Giardia* infections. These studies indicate that prior infections induce an immune response, and persons with an immune response from prior *Giardia* infection(s) have a reduced risk of illness when a subsequent *Giardia* infection occurs. Although the immune response may not result in reduced risk of subsequent *Giardia* infections, subsequent infections are more likely to be asymptomatic.

1. Epidemiological Data Supporting Acquired Immunity

The epidemiology of giardiasis in developed countries indicates that persons episodically exposed to *Giardia* cysts are more likely to suffer symptoms and illness. These persons include travelers to certain locales, backpackers, expatriates, case-contacts, and persons exposed during waterborne outbreaks (Janoff and Smith, 1990). In contrast, residents with recurrent exposure to *Giardia* are commonly asymptomatic (Janoff and Smith, 1990).

High rates of exposure to and high rates of carriage of *Giardia* are associated with low rates of symptomatic illness, and persons infected with *Giardia* in developing countries are usually asymptomatic (Gilman et al., 1988; Zaki et al., 1986; Walia, et al., 1986; Nacapunchai et

al., 1986). In developing countries, exposure to *Giardia* begins early in life (Zaki et al., 1986; Gilman et al., 1985), high rates of *Giardia* carriage are present in all age groups (Zaki et al., 1986; Janoff et al.,1990). Asymptomatic reinfection occurs at a high rate (Gilman et al., 1988). For example, in Egypt *Giardia* is frequently detected in healthy subjects. Immunity to *Giardia* may be particularly important in recurrently exposed persons who, though often infected, are infrequently ill. In Bangladesh, symptoms are reported to occur in 86% of infants newly infected with *Giardia* but in only 4% of infected mothers (Janoff and Smith, 1990). In Thailand, most school children and adults, repeatedly exposed to *Giardia*, are asymptomatic (Chavalittamrong et al., 1978; Walia et al., 1986).

Studies have also suggested that persons who travel to *Giardia* endemic areas from relatively non-endemic areas carry a high risk of developing giardiasis. Speelman and Ljungstrom (1986) reported that the annual incidence of giardiasis was 12% among 251 expatriates in Bangladesh with 37% of infected persons developing diarrhea. Giardiasis was more common among the newcomers and children less than 10 years old. Epidemiological studies also suggest that new settlers or travelers visiting areas where *Giardia* is endemic are more likely to develop symptomatic illness, possibly related to the lack of immunity from prior infections (Rabbani and Islam, 1994). Age which may be a measure of prior exposure or infection has also been related to a reduced risk of giardiasis. If increasing age is related to the likelihood of prior *Giardia* infection and persons develop immunity, then older people would be expected to be less susceptible to illness or severe illness from infection. The attack rate for giardiasis has been found to decline with increasing age supporting this hypothesis (Farthing 1989).

Since infection occurs at an early age and reinfection is common in developing countries, it has been argued that a vaccine to *Giardia*, if developed, would have only limited application in these countries (Janoff and Smith 1990). Immunity to giardiasis likely develops at a early age, possibly before a vaccine could be effectively administered (Gilman, 1988). Based on this

argument a vaccine would be most helpful for persons infrequently or episodically exposed to *Giardia*, such as travelers and military personnel (Janoff and Smith, 1990).

Persons in developing countries are at a low risk of symptomatic giardiasis and also have high levels of parasite-specific antibody (Gilman et al., 1985; Nacapunchai et al., 1986; Miotti et al., Janoff et al., 1988a). Healthy homosexual men also have a high frequency of asymptomatic infection in association with increased levels of *Giardia*-specific antibody (Janoff et al., 1988a).

Specific evidence for acquired immunity to giardiasis has been found in several epidemiological studies in developed countries. In Colorado, visitors were found more likely to experience symptomatic giardiasis than long-term residents, and residents who lived in the area for more than two years had a lower attack rate for illness during a waterborne outbreak than short-term residents (Wright, 1977; Istre 1984). The presumption is that long-term residents were repeatedly exposed to *Giardia* cysts through drinking water and perhaps other sources, and they developed an immunity which protected them, when reinfected, from illness or less severe illness. Visitors, with fewer prior exposures to *Giardia*, did not develop this immunity and were, therefore, more likely to become ill when infected. Another study found that persons with prior diagnosed giardiasis were at a lower risk of giardiasis during a subsequent exposure. In a community that experienced two waterborne giardiasis outbreaks separated by a five year period, individuals infected during the first outbreak were at significantly lower risk during the second outbreak (Isaac-Renton, 1994; Isaac-Renton et al., 1994). See Section F, Chapter III for a further discussion of these outbreaks.

2. Breast Milk and Breast Feeding Reduces the Risk of Giardiasis

The role of breast-feeding in preventing enteric infections in young children is now been well recognized. However, in these studies it is often difficult to distinguish the role of antibodies in the breast milk versus other anti-parasite effects of milk. Secretory IgA (sIgA) to

Giardia has been demonstrated in the breast milk from women in Bangladesh (73%) and Mexico (77%), indicating a high incidence of the disease in these populations (Islam et al., 1983; Miotti et al., 1985). Infants in these areas may thus acquire antibodies by way of breast milk (Islam, 1983, Miotti, 1985). An experiment in which children were exclusively fed breast milk resulted in a very low rate of *Giardia* infection, and the infections which occurred were mostly asymptomatic (Rabbani and Islam, 1994). This may explain why giardiasis is uncommon in infants younger than 6 months, during which period most children are usually breast-fed.

The role of breast milk in the prevention of giardiasis has been investigated in both animals and humans. Suckling mice are protected against *G. muris* when they are fed milk from immune mothers containing specific IgA antibodies to *Giardia*. It has also been shown that *Giardia* is rapidly killed by exposure to normal human milk in vitro. However, this killing effect is not mediated through antibodies; rather, the effect is related to the exposure of the organisms to an enzyme, bile salt-stimulated lipase. Similar observations are reported by Reiner et al. (1986) who showed that normal human milk kills *Giardia* trophozoites in vitro and that this effect is mediated by the release of free-fatty acids from milk triglycerides by the action of the bile-stimulated lipase on human milk. Thus, from the public health point of view, children who are not breast-fed are at a higher risk of developing *Giardia* infections, particularly in the endemic areas with high levels of fecal contamination of the environment, and are most at risk of severe consequences of giardiasis.

3. Increased Giardiasis Risks in Immunosuppressed Populations

Investigators have found that athymic mice exposed to *Giardia* are at an elevated risk of suffering from chronic infections whereas exposed immunocompetent mice appear to clear the infection and become immune to reinfection (Roberts-Thomson et al. 1976). Implantation of the thymic tissue in athymic mice reduced the number of mice chronically infected with *Giardia* (Roberts-Thomson and Mitchell, 1978). Other studies have indicated that most strains of mice

appear to be resistant to reinfection after clearing a primary infection whereas T-deficient mice have prolonged carriage and fail to produce local antibody (Janoff and Smith, 1990).

Based on evidence from the studies of *G. muris* infection in athymic mice, the role of T lymphocytes in the host response to *Giardia* was investigated in normal mice. During infection with *G. muris*, the number of Peyer's patch lymphocytes may double, although the ratio of T helper to T suppressor lymphocytes (>5:1) does not change (Clark and Holberton, 1986). When these mice are depleted of T helper cells they develop chronic infection, whereas mice depleted of T suppressor cells or deficient in natural killer cells clear their infection (Heyworth et al., 1986, 1987). This indicates that T helper cells may play an important role in the ability of the murine host to clear *Giardia*. Recently, T helper cells were reported to contribute, possibly as 'switch' cells, to the sequential change in Peyer's patch B cells from IgM-bearing during the first week of infection to IgA-bearing B cells during the second week of infection (Clark and Holberton, 1986). Although intriguing, the role of T helper cells in the local immune response to *G. muris* remains speculative at this time. (Janoff and Smith, 1990).

At the level of the intestinal mucosa, the host response to *Giardia* is initiated by the presentation of parasite antigen to T lymphocytes. In vitro studies of murine, rabbit and human macrophages confirm that macrophages are capable of phagocytosing *Giardia* trophozoites. The ability of the parasite to invade the intestinal mucosa, and the presence of macrophages in the lamina propria, would facilitate contact between the parasite and antigen-presenting cells. In addition to antigen presentation, effector cell function by monocytes-macrophages contributes to host defense mechanisms against many pathogens. The ability of resident macrophages and local blood monocytes in the lamina propria to kill *Giardia* could provide an important defense mechanism against invading trophozoites. In this regard, three reports indicate that human mononuclear phagocytes are capable of killing *Giardia*, possibly by the products of oxidative metabolism (Hill and Pearson, 1987). Aggarwal and Nash (1986) did not confirm these findings.

Results of animal studies conducted to understand the importance of immunological responses to infection indicated that when anti-*Giardia* antibodies are transferred to immunoglobulin deficient mice the risk of developing chronic infection is not reduced (Snyder et al., 1985). The authors concluded that immune responses in the intestine tract are required for protection against illness. In vitro work (Goka et al 1986, Nash et al., 1987 Janoff et al., 1988b) found that anti-*Giardia* IgM antibodies were capable of sensitizing *Giardia* for complement lysis. These findings support the role of secretory anti-*Giardia* antibodies in clearing infection.

The findings of a high risk of chronic infection in immunocompromised animals are consistent with findings from human studies. People with hypogammaglobulinemia are at high risk of chronic infection (Janoff and Smith, 1990). Children with chronic diarrhea and giardiasis have an increased incidence of hypogammaglobulinemia (Perlmutter et al., 1985). The presence of circulating anti-*Giardia* antibody may not be sufficient protect immunocompromised persons from infection, and one study (Smith et al., 1982) reported recurrent giardiasis in some human patients with high titers of anti-trophozoite antibodies.

Cellular immune response to infection occurs in the intestinal mucosa. Varying degrees of histological change following infection have been observed. The accumulation of inflammatory cells in the small intestinal mucosa of infected persons suggests that cellular response may be an important component of the host response to infection. As previously indicated, T cells appear to play a significant role in clearing infection. Human peripheral blood mononuclear cells were spontaneously cytotoxic for *G. lamblia* trophozoites, and the observed cytotoxicity was a host-defense mechanism directed against extracellular protozoa in general (ICAIR, 1984). Where there was both giardiasis and malabsorption, the lymphocyte counts were significantly higher than those in the controls, as well as in those patients with giardiasis and normal absorption. In most cases, the intraepithelial lymphocyte counts declined after giardiasis treatment.

4. Measuring Epidemic and Endemic Infections in Humans

A variety of techniques have been developed for improving the detection of *Giardia* cysts in stool specimens. These include an ELISA assay which improves the microbiologist's ability to distinguish cysts from other similar sized particles. Since *Giardia* is a common intestinal infection among children, stool surveys are a feasible method for estimating the prevalence of infection. Approximately 7% of children in diapers were found to be infected in one survey (Frost et al., 1983).

a. Anti-*Giardia* Antibodies in Sera

A variety of methodologies have been employed for detection of anti-*Giardia* antibodies in serum, including immunodiffusion, hemagglutination, immunofluorescence, and ELISA (ICAIR, 1984; Sullivan et al., 1987). Use of serum antibody tests to identify infections have several limitations. Serological assays have a reduced clinical value since the serological response may take several weeks to appear. There is also no agreement on whether serological tests can distinguish an active infection from a recently cleared infection, and it is unclear how to interpret the results of serological tests performed on chronically infected persons.

Serological tests have advantages for epidemiological studies. Sera tend to be easier to obtain than are stool samples. Also, if the serological response is longer lived than the infection, it may be possible to better distinguish populations with a low versus high prevalence of infection. Unfortunately, the current serological methodologies have significant limitations. Most of the ELISA tests are based on serological reaction to all proteins in the parasite. Since *Giardia* is a complex organism with many potential human antigens, it is likely that much of the response measured by the ELISA test is non-specific, resulting from serological reactions to antigens which are shared by *Giardia* and a variety of other organisms. Western blot tests, which look for serological response to antigens separated by weight, are likely to be more specific, but

there is currently only limited experience in using Western blots for serological surveys of anti-*Giardia* antibodies in human populations.

The pre-1984 literature on detection of anti-*Giardia* antibodies in serum has been reviewed (ICAIR, 1984). Elevated levels of serum immunoglobulin IgG, IgM and IgA were reported in patients with giardiasis (ICAIR, 1984). There seemed to be a consensus that levels of serum IgE and probably IgD in giardiasis patients may not differ from those of normal controls. Unfortunately, various studies have not reported consistent findings of elevated immunoglobulins G, A and M, due in part to the limitations of the assay used and in part to different populations surveyed. In theory, the early immune response to *Giardia* infection was believed to be restricted to IgM, followed later by IgA and IgG (ICAIR, 1984), however not all serological studies have found elevated levels of IgM in recently infected persons (Birkhead et al., 1989).

Nash et al. (1987) reported specific anti-*Giardia* serum IgM responses in 100% of human volunteers experimentally infected with human-source *Giardia*. Such responses were observed within two weeks of infection in 70% of acute episodes, but were low in chronically infected and rechallenged individuals. The IgM detected by the ELISA procedure may have limited diagnostic usefulness in chronic or repeated infections.

Problems associated with attempts to diagnose giardiasis by testing a single serum sample for total human globulin IgG antibodies have been described. Although some authors believe that serologic procedures are reliable for retrospective diagnosis of symptomatic patients, they are unreliable in asymptomatic patients due to nonspecific antibody titers caused by intestinal parasites other than *Giardia*. Jokipii et al. (1988) concluded that their ELISA procedure, which employed *Giardia* cysts as antigen, was insufficiently sensitive and specific because the anti-*Giardia* antibody titers in the patient population were indistinguishable from titers in healthy controls.

Haralabidis (1984) demonstrated that sera from patients with microscopically-proven *Giardia* infection reacted by ELISA with a variety of parasite antigens indicating either co-infection or a cross-reaction between anti-*Giardia* antibodies and non-*Giardia* parasite antigens (a lack of specificity). Jokipii et al. (1988) concluded that sera from most people contains antibodies which cross-react with *Giardia* cysts, and that these antibodies may be induced by immunogens other than *Giardia*.

In 1986 a method was published for detection of relatively short-lived IgM antibodies. Results might be more clinically relevant, in that they would more likely indicate the cause of a patient's present symptoms. Goka et al. (1986) concluded that serum IgG responses were not helpful in distinguishing active from past *Giardia* infection because they were relatively long-lived.

Taylor and Wenman (1987) and Heyworth and Pappo (1990) reported that convalescent sera of most patients with giardiasis contain antibodies directed against a specific 30/31 kDa *G. duodenalis* antigen. Serologic assays using this purified antigen might prove to be more sensitive and specific than assays employing a mixture of antigens, such as crude trophozoite or cyst preparations. Other authors have suggested that high molecular weight antigens (155 and 170 kDa) or a 57 kDa antigen are predictive of infection (Char, 1991). These approaches have considerable merit, since cross-reactions are likely reduced in an assay which only examines serological response to selected antigen groups rather than to the entire parasite. Additional research is needed to confirm these findings and apply these approaches in population studies, especially during outbreaks. This may result in a highly sensitive and specific marker of infection which could be used to compare endemic levels of infection in various communities.

b. Anti-*Giardia* Antibodies in Saliva

Results of an immunofluorescence procedure to detect *Giardia*-specific antibodies in saliva were compared to serum titers. Results suggest that saliva may be a more suitable specimen than serum for detection of anti-*Giardia* antibodies in patients with giardiasis but more studies are needed to confirm these results (and Pickering, 1990). Saliva tests have an intuitive appeal since they can be applied to studies of children and do not require drawing blood. Rosales-Borjas et al. (1998) studied the secretory immune response during natural *Giardia* infections in 24 patients and were able to demonstrate a secretory IgA response in their saliva that was not present in healthy individuals.

c. Anti-*Giardia* Antibodies in Intestinal Secretions

Nash et al. (1987) described humoral and intestinal fluid IgA responses which were detected at the same time as intestinal IgA responses; however only 50% of the patients demonstrated significant rises in *Giardia*-specific intestinal IgA antibodies and the presence of these intestinal antibodies did not prevent *Giardia* infection in these patients.

5. Mechanisms of Protection

The role of humoral antibodies in protecting against *Giardia* infections is poorly understood. Although it has been reported that anti-trophozoite antibodies including IgG, IgM and IgA, are produced in the body in response to *Giardia* infections (Miotti et al., 1985; Goka et al., 1986), it is not known whether these antibodies are immunologically relevant to clinical disease. In an epidemiologic study in Bangladesh, Islam et al. (1983) reported that 45% of asymptomatic mothers who had serum IgG antibodies against *Giardia* excreted the cysts in their feces. Similar findings in Peru were reported by Gilman et al. (1985) who observed that 39% of persons with serum IgG antibodies excreted the organisms. These observations suggest development of a partial immunity which protects against the disease but not against infection. In Bangladesh, young children especially lacked large amounts of antibodies in their serum and

the first infections were usually symptomatic (Islam et al., 1983). However, conflicting evidence was reported by Miotti et al. (1986) in Peruvian children.

Regarding the role of antibody in asymptomatic infections, antibody levels may either predict a protective effect or serve as a marker of infection. High levels of serum antibody to *Giardia* are observed in developing countries where endemic levels of *Giardia* infection are high. These countries also have very low levels of symptomatic giardiasis (Janoff and Smith 1990). In animal studies, anti-*Giardia* secretory IgA levels are predictive of the clearance of infection, and serum IgA levels appear to predict secretory IgA levels (Conley and Delacroix, 1987). This suggests that serum IgA levels should be predictive of clearance of infections. Evidence for a relationship between serum IgA levels and infection in human populations is provided by Nash (1987) from his experimental infection of 15 healthy volunteers. High levels of infection were found with one strain and seroconversion for IgG, IgA and IgM among persons with confirmed infections (Nash 1987). Rechallenge and infection resulted in less illness than the initial challenge, but the number of individuals involved in the rechallenge was very small.

6. Summary of Evidence for Immunity

There is variability in the humoral response to *Giardia* infection. Some patients with symptomatic infections fail to develop sufficiently high antibody levels for results to be called positive. (Engelkirk and Pickering, 1990). In some patients, levels of anti-*Giardia* IgG antibodies remain elevated long after the infection appears to have been eradicated. No sero-diagnostic procedure has been reported that is capable of distinguishing asymptomatic from symptomatic infection. Despite these shortcomings, serologic assays have proven to be of some value in epidemiologic investigation of giardiasis (Miotti et al., 1986). The presence of anti-*Giardia* antibodies may indicate either past or present infection with *Giardia*, whereas the presence of *Giardia* antigen in stool specimens indicates current infection. The specificity of antigen detection assays may be significantly improved by assays based on certain antigens

groups (30/31 kDa, 57 kDa and high molecular weight antigens). Additional research is needed to evaluate these markers under both controlled and field conditions.

F. Nonspecific Defenses Against Human *Giardia*

The severity of symptoms and the duration of *Giardia* infection are extremely variable. This was reflected in a study of experimental human infections with a single *Giardia* strain (Nash et al., 1987) where the severity and duration of giardiasis bore no apparent relationship to the magnitude of the serum or secretory antibody responses (Nash et al., 1987). Gillin et al. (1990) feel the variability in the severity of giardiasis symptoms may be due in part to trophozoite interactions with non-immune elements of intestinal milieu.

The upper human small intestine colonized by *Giardia* is a complex and ever-changing environment, which is normally inhabited by relatively types of few microbes (Gillin et al., 1990). *Giardia* trophozoites in the intestinal lumen are exposed to fluctuating pH and concentrations of bile, nutrients, digestive enzymes and their products. The intestinal tract has a unique system of defenses which is less well understood than circulating defenses. Secretory defenses may be produced locally, secreted into the duodenum, or ingested--as by breast-fed babies. This system has both immune (secretory antibody) and non-immune components (lysozyme, lactoferrin, lipases, many of which are found in both breast milk and intestinal fluid (Gillin et al., 1990). Certain intestinal fluid factors might be toxic to the parasites, while other factors such as mucus might protect them (Gillin et al., 1990)

Gillin et al., 1990 and others (Hernell et al., 1986; Rohrer *et al.*, 1986) have shown that *Giardia* trophozoites are killed in vitro by normal human milk and by human intestinal fluid (Das et al., 1988). Products of lipolysis such as unsaturated free fatty acids (FFA) (Gillin et al., 1990; Reiner et al., 1986; Hernell et al., 1986; Rohrer et al., 1986), lysophospholipids or monoglycerides generated by lipases in human milk (Reiner et al., 1986) or intestinal fluid such

as mucus and bile salts which bind lipolytic products protect trophozoites from killing by human milk (Zenian and Gillin, 1987) and intestinal fluid (Das et al., 1988), as well as free fatty acids. (Reiner et al., 1986). Bile also contains major growth factors for *Giardia*. This may help explain how *Giardia* specifically colonizes the human small intestine.

Symptomatic and asymptomatic infections may result from the interaction of several different aspects of the host-parasite biology of giardiasis. Intestinal conditions that are conducive to growth of *Giardia* may vary between individuals or may be different in some populations. Some patients with giardiasis and malabsorption show bacterial colonization of the small bowel, which may predispose *Giardia* to express virulence factors (Janoff and Smith, 1990).

Because cellular immune responses to *Giardia* occur at the level of the intestinal mucosa, it is important to appreciate the spectrum of mucosal inflammatory changes that may accompany giardiasis. In the mouse, trophozoites colonize the proximal small intestine, attaching to the mucosa preferentially near the bases of villi, at the edges of Peyer's patch follicles, and less frequently on villous tips. Although *Giardia* may be taken up by the specialized membranous 'M' cells overlying Peyer's patches, trophozoites appear not to attach preferentially to these cells, whose function is to transport luminal antigens and microorganisms to the underlying lymphoid cells. Infrequently, trophozoites may invade the mucosal epithelium and penetrate the lamina propria, possibly coming into direct contact with lymphoid cells. However, the frequency of tissue invasion is unknown, because intestinal biopsies are not routinely performed in persons with giardiasis.

Although the mucosal histology of the proximal small intestine of persons infected with *Giardia* is frequently normal, varying degrees of histological change have been observed during giardiasis. The histological changes include infiltration of polymorphonuclear leukocytes into the epithelium and mononuclear leukocytes into the lamina propria, development of shortened

villi, loss of the brush border, damage to epithelial cells, and an increase in epithelial cell mitosis. Rarely, infection with *Giardia* may be associated with total villous atrophy, dense mononuclear cell infiltration, and flattening of the epithelial cells, changes that resemble celiac sprue. These findings indicate that the parasite is capable of eliciting an inflammatory cell response of varying degree in infected subjects.

The accumulation of inflammatory cells in the small intestinal mucosa of infected persons suggests that cellular responses may contribute to the host response to *Giardia*. The presence of recurrent giardiasis in some patients with high titers of anti-trophozoite antibodies supports the notion that circulating antibodies alone are not protective against the parasite. Investigations using an experimental animal model of giardiasis provide evidence that lymphoid cells, particularly T lymphocytes, contribute to host responses to the parasite. In athymic mice, which are deficient in both circulating T lymphocytes and Peyer's patch helper T lymphocytes (Heyworth et al., 1985), inoculation with *G. muris* cysts results in chronic infection with large numbers of trophozoites. In contrast, immunocompetent mice clear the parasite and may develop resistance to reinfection, implicating a role for T lymphocytes in the pathogenesis of chronic *G. muris* infection.

G. Variation in Pathogenicity

Variability in the clinical response of the host to infection with *Giardia* may also be due, in part, to differences in the pathogenicity of various strains of the parasite. Variation in the host response to different strains has been identified in both humans (Nash et al., 1987) and animals (Aggarwal and Nash, 1987) but strain differences only partly explain the variable host response to the parasite. (Janoff and Smith, 1990). The variable pathogenicity among strains of *Giardia* may also reflect differences in certain parasite antigens. In vitro demonstration of antigenic variation, both spontaneous and in response to immunologic selection, suggests a possible

mechanism by which *Giardia* survives chronically in the intestine, despite the presence of an active immune response (Jannoff and Smith, 1990).

H. Chronic Conditions

Chronic patients often present with recurrent, persistent, brief episodes of loose, foul-smelling stools which may be yellowish and frothy in appearance, frequently accompanied by distension, foul flatus, anorexia, nausea, and uneasiness in the epigastrium (ICAIR, 1984). With chronic giardiasis, there is increased frequency of constipation and upper gastrointestinal tract complaint (ICAIR, 1984). In some cases, these symptoms may persist for years, and in the majority of cases, the parasite and symptoms disappear spontaneously (ICAIR, 1984). Among 65 giardiasis cases encountered in an urban private practice outpatient setting, the mean duration of symptoms was reported to be 1.9 years, and of 38 (58%) patients who exhibited chronic symptoms for six months or longer, the mean duration of symptoms was 3.3 years (ICAIR, 1984). Hoskins et al. (ICAIR, 1984) reported one patient who had a sprue-like syndrome with IgA deficiency, vitamin B_{12} malabsorption, and recurrent diarrhea which persisted for 25 years. The episodes consisted of 15 to 20 foul-smelling stools daily, anorexia, vomiting, nausea, and abdominal cramps. Histological examination revealed abnormal villus architecture.

There is usually no extra-intestinal invasion when *Giardia* trophozoites infect the small intestine, but reactive arthritis may occur, and in severe giardiasis duodenum and jejunal mucosal cells may be damaged (Benenson, 1995). Some symptomatic patients suffer from chronic diarrhea, steatorrhea, and malabsorption of fats or of fat-soluble vitamins. A small amount of fat in feces is not unusual, but when daily losses are greater than 7 grams, the condition is classified as steatorrhea (Hall, 1994). Prolonged malabsorption of fat and its excretion in stools could lead to a significant loss of potential dietary energy, especially as a result of chronic infection. This will be of greater consequence for young children since they have greater requirements for energy than adults and have small stomachs. The energy density of the diet and its efficient absorption

are important, and persistent malabsorption of fat due to *Giardia* could lead to protein-energy malnutrition (Hall, 1994). A loss of appetite is a commonly reported symptom, and nausea or vomiting, abdominal cramps and bloating may occur (Hopkins and Juranek, 1991). All of these symptoms are likely to contribute to reduced food intake (Hall, 1994). Studies have also shown malabsorption of micro nutrients, especially vitamins A and B_{12}, in infected persons (Hall, 1994).

The association between *Giardia* infection and ocular changes has previously been described, but until recently, no large scale studies had been conducted. Corsi et al. (1998) evaluated ocular manifestations in 141 Italian children with current and past giardiasis and 300 children without giardiasis. Salt and pepper retinal changes were diagnosed in 20% of the children with giardiasis (mean age was 4.7 years) and in none of the children without giardiasis. These findings suggest that asymptomatic, non-progressive retinal lesions may be common in young children with giardiasis, and the risk did not seem to be related to the severity of infection, its duration, or use of metronidazole but may reflect a genetic predisposition (Corsi et al., 1998).

Lactose intolerance is common during active infection and may persist following anti-*Giardia* treatment, especially in ethnic groups predisposed to lactase deficiency (Wolfe, 1990).

II. Summary

Progress has been made in understanding the biology of *Giardia*. However, there is still no adequate explanation for the wide clinical spectrum of giardiasis which ranges from asymptomatic infection to acute self-limiting diarrhea to more persistent chronic diarrhea, which sometimes fails to respond to therapy; the mechanisms by which *Giardia* produces diarrhea and malabsorption and the key immunologic determinants for clearance of acute infection and development of protective immunity also remain poorly understood.

Symptomatic giardiasis, although less common than asymptomatic infection, occurs frequently and results in diarrhea, flatulence, abdominal pain, weight loss, and vomiting. Severe disease may result in malabsorption or growth retardation, but rarely death. Diarrheal symptoms have been related to abnormal villus architecture. Chronic giardiasis appears to be infrequent, but when it occurs, may persist for years. The precise mechanism of giardiasis responsible for its pathology and symptomatology in humans remains unknown at present.

As with all diarrheas, fluid replacement is an important aspect of treatment; anti-giardial drugs are also important in the management of giardiasis. Chemotherapeutic agents used for treatment of giardiasis include metronidazole, tinidazole, quinacrine, furazolidone, albendazole, and ornidazole. Various doses and treatment periods are recommended for each drug. The drugs may have different effectiveness in their ability to clear *Giardia*; drug resistance and relapses may occur, and the drugs have side-effects that should be considered. Paromomycin has been used to treat giardiasis in pregnant women, but the cure rate may be low.

Data on the nature of human immune response to giardiasis are somewhat limited, but there are indications that both humoral and cellular responses are present. Most subjects infected with *Giardia* produce detectable levels of anti-parasite antibodies. However, the role of specific antibody to *Giardia* in determining the host's clinical response to infection has not been delineated. Is the presence of specific antibody more frequently associated with symptomatic *Giardia* infections or asymptomatic infections (Janoff and Smith, 1990)?

When *Giardia* organisms enter the small intestine, local factors such as bile salts, intestinal mucus, or the presence of other organisms enhance or inhibit their initial growth. Intestinal enzymes, which may induce the expression of surface lectins that mediate mucosal adherence, facilitate colonization (Lev et al., 1986). Once infected, the individual may become ill, depending on the virulence of the strain or the presence of pre-existing immunity. In the absence of prior immunity, interaction of trophozoites with intestinal macrophages may initiate

an immune response. This nonspecific interaction may eliminate the parasite via cytotoxic mechanisms, or macrophages may process trophozoite antigens leading to the induction of a specific antibody response. These antibodies may be directly cytotoxic for the parasite or promote antibody-dependent cytotoxicity with granulocytes, and possibly monocytes. This integrated immune response results in clearance of the organism and resolution of symptoms. However, this sequence of events does not explain the chronic, asymptomatic excretion of the parasite observed in recurrently exposed persons.

Although one species of *Giardia* is believed to infect humans, the epidemiology of giardiasis is complicated by an apparent genetic heterogeneity in this species. Differences in virulence, pathogenicity, infectivity, growth, drug sensitivity, and antigenicity have been reported. In endemic areas where extensive heterogeneity exists, mixed infections with more than one genotype may occur.

VI. *GIARDIA* RISK ASSESSMENT

A. Risk Assessment Paradigms

Risk assessment is the qualitative or quantitative characterization and estimation of potential adverse health effects associated with exposure of individuals or populations to hazards (materials or situations, physical, chemical and or microbial agents) and is a component of risk analysis. Risk analysis also includes risk management and risk communication (Table VI-1). This section will address risk assessment and to some extent risk management.

Table VI-1 Components of Risk Analysis

Risk Assessment	The qualitative or quantitative characterization and estimation of potential adverse health effects associated with exposure of individuals or populations to hazards (materials or situations; physical, chemical and or microbial agents).
Risk Management	The process for controlling risks, weighing alternatives, selecting appropriate action, taking into account risk assessment, values, engineering, economics, legal and political issues.
Risk Communication	The communication of risks to managers, stakeholders, public officials, and the public, includes public perception and ability to exchange scientific information.

Source: Hoppin, 1993

Before 1991, risk assessment methods following the National Academy paradigm (NRC, 1983) of hazard identification, dose-response, exposure assessment, and risk characterization were only used on a limited scale for assessing risks of waterborne pathogens (Haas, 1983; Rose et al., 1991b; Rose and Gerba, 1991; Regli et al., 1991). The National Academy of Sciences has suggested that risk assessment and risk management be kept separate (NRC, 1983), but in reality the integration of risk management and risk assessment is seen as a necessary requirement in the development of a workable framework. Regulatory agencies are now attempting to develop the best approach for undertaking and using microbial risk assessment for policies that will improve water quality, food safety and public health. The EPA (1989a) first used risk assessment based

on dose-response models for the development of the Surface Water Treatment Rule (SWTR). The National Institute of Public Health and Environmental Protection in the Netherlands has also used formal risk assessment procedures for waterborne microorganisms (Teunis et al., 1994).

A national committee established by the EPA in 1995 developed a framework for pathogen risk assessment describing how to conduct a risk assessment, the type of data needed, and the available information (ILSI et al., 1996). The framework attempts to integrate risk management and microbial risk assessment. The committee was composed of a multi-disciplinary group of scientists from the fields of epidemiology, medicine, microbiology, water treatment, food safety, chemical risk assessment and public policy. The initial step in the framework is problem formulation. Articulated in this step is the information needed by managers to make decisions including the types of risks and priorities that are to be addressed.

The risk assessment itself is defined by an analysis involving the characterization of both exposure and health. This leads to an estimate of risk and risk management options. The analysis phase of the risk assessment considers:

1. human health effects (symptomatic and asymptomatic infection, severity and duration of illness, medical care and hospitalization, mortality, host immune status, susceptible populations);

2. dose-response modeling based on clinical and epidemiological data;

3. exposure analysis (vehicle of infection, amount and route of exposure and whether it is acute or recurring, demographics and other characteristics of persons exposed);

4. occurrence assessment (analytical methods, quantitative measures of the pathogen in the vehicle of exposure and its frequency, spatial and temporal variation, regrowth, die-off, and transport).

Haas (1983) first estimated quantitative risks associated with microbiologically contaminated drinking water using dose-response data from exposure of humans in clinical experiments. Several

mathematical models were evaluated to determine the model that best described the probability of infection from existing dose-response data. For waterborne viruses, Haas (1983) found that a beta-Poisson model best described the probability of infection, and this model was used to estimate annual and lifetime risks for infection, clinical disease, and mortality associated with hypothetical levels of viruses in drinking water.

Rose et al. (1991b) have used an exponential model to evaluate risks of *Giardia* infection from estimated exposures to *Giardia* in drinking water. Drinking water exposures were obtained from survey data describing the occurrence of *Giardia* in polluted and pristine water sources and considering average removals and inactivation of cysts with various types of water treatment. The same approach was used in the development of the SWTR where performance-based standards for the control of *Giardia* were developed to meet the EPA's recommended public health goal of no more than one *Giardia* infection per 10,000 persons from drinking water exposures (U.S. EPA, 1989a). The EPA felt that this goal could be maintained by achieving 99.9% reductions of *Giardia* cysts through filtration and disinfection in all water systems.

B. Health Effects

The anticipated health effects are defined by the specific microbial agent, host characteristics, the spectrum of symptoms, and pathology associated with the infection. The types of clinical outcomes may include asymptomatic infection, acute disease, chronic disease, hospitalization, or death, and outcomes may differ for sensitive subgroups. The pathogenicity and virulence of the microorganism itself is of interest as well as the full spectrum of human disease which it can cause. The host's response to the microorganism in regard to immunity is also of concern. Information from endemic epidemiological studies, epidemic disease or outbreak investigations, case-studies, hospitalization studies and other clinical is needed to complete this step in the risk assessment.

The transmission of disease and the vehicle of infection is often microbial specific (i.e. rabies, malaria, influenza), but this is not the case with *Giardia*, which is transmitted by the fecal-oral route. Water is only one of several modes of transmission.

The human health effects and epidemiology of giardiasis are described in detail in Chapters III and V. Information about the prevalence of infection and the importance of contaminated drinking water is found in Chapters III and V. An examination of the waterborne outbreak and prevalence data can provide information about the relative importance of waterborne transmission of *Giardia* compared to other fecal-oral pathogens that can also be transmitted by contaminated water. Based on these data, *Giardia* is a waterborne pathogen of primary importance in the United States. *Giardia* is the most frequently identified etiologic agent causing waterbome outbreaks in public water systems in the United States, and it is the most frequently identified parasite in national surveys of stool specimens where its prevalence ranges from 4.0% up to 12% depending on the year and state. Outbreaks summarized in Chapter III, Section G (Table III-7) show that *Giardia* is also transmitted by accidental ingestion during swimming and other water recreational activities. Foodborne outbreaks of giardiasis have also occurred but are much less frequently reported than waterborne outbreaks. Other important transmission routes and risk factors include person to person transmission, travel to developing countries, homosexual activities, and day-care center use.

The quantitative description of the health effects include the types, severity, and duration of the illness. Illness factors are summarized in Table VI-2, and further information about them can be found in more detail in other chapters of this document. Associated health care costs and costs associated with days lost from work have been quantified, but these data are limited. In 18 reported waterborne outbreaks of giardiasis, hospitalization data were reported -- 60 persons were hospitalized among a total of 13,239 cases for a 0.5% ratio. Using estimates of 4,600 hospitalized cases or 2 hospitalizations per 100,000 persons per year (Lengerich et al., 1994) and the computed hospitalization ratio (0.5%), as many as 593,400 cases of giardiasis are estimated

to occur annually. Bennett et al. (1987) has suggested that 60% of giardiasis in the United States is waterborne and estimated that 120,000 cases of waterborne giardiasis may occur each year, however, this estimate is not based on epidemiological data. Mortality associated with *Giardia* infections is rare (Bennett et al., 1987). Evidence in both animals and humans suggests that the immunocompromised may be at a greater risk of acquiring a chronic giardiasis infection with chronic diarrhea (Chapter V). An immune response is found after infection and may confer protection; epidemiological studies (Isaac-Renton, 1994; Isaac-Renton et al, 1994) suggest that immunity for *Giardia* may last for five years.

Table VI-2 Selected Health Factors Considered in Assessing Waterborne *Giardia* Risks

Health Effect	Quantitative Assessment
Symptoms	Diarrhea with loose, foul-smelling stools that are greasy, frothy or bulky; abdominal cramps, bloating, nausea, decreased appetite; malaise and weight loss in the majority of patients. Illness lasts in untreated individuals on average about 1 week with infection lasting 6 weeks. Chronic diarrhea can last an average of 1.9 years. Chronic outcomes include failure to thrive, urticaria, and reactive arthritis.
Asymptomatic infection	25% to 75%
Waterborne outbreaks in U.S.	Associated with 32% of all drinking water outbreaks, from 1971 to 1994, and 70% of all recreational outbreaks from 1991 to 1994 relative to other known etiologic agents
Prevalence of infection	Detection of the cyst varies world-wide between 2 and 5% in diarrheal stools in industrialized countries. Found in 4% to 12% of all diarrheic stools examined for parasites in U.S.
Prevalence of waterborne illness	593,400 cases annually based on hospitalized cases and hospitalization ratio. 60% of cases may be waterborne; estimated 120,000-356,000 waterborne cases annually but estimate not from epidemiological studies.
Immune status	Immunity clears infection and may provide some protection up to 5 years. 10% of population with IgA deficiencies may suffer chronic infections.
Sensitive populations	Immunocompromised greater sensitivity to chronic outcomes , however this has not been quantified.
Severity	Annually 2 hospitalizations/100,000. Hospitalization ratio = 0.5%. Mortality = 0.0001%
Costs associated with illness	One week of illness estimated to cost $250 to $500. This does not include treatment costs for those with chronic cases.

Methods for diagnosis	Available, routinely in use only when requested that specimen be sent to ova and parasite laboratory. Can be difficult to diagnose (3 stool submissions), chronic infections detected through intestinal biopsies.

C. Dose-Response Modeling

Dose-response studies provide information which can be used to mathematically characterize the relationship between the administered dose and the probability of infection or disease in an exposed population. Natural routes of exposure are used in clinical studies; that is, the direct ingestion by volunteers in a controlled experiment. Both disease and infection are measured. Epidemiological studies can also provide similar information, but the dose or exposure may not be well known. Methods used to determine the number of microorganisms in a given dose are those routinely used in the laboratory (e.g., direct microscopic counts of *Giardia* cysts similar to those used to detect the cysts in environment samples). However, for *Giardia* studies, this means non-viable cysts viewed microscopically will be included in estimating the administered dose.

One of the more controversial areas surrounding microbial modeling is the assumption that a single organism (one *Giardia* cyst) can initiate a human infection. The early literature suggested that many microorganisms were needed to act cooperatively to overcome host defenses in order to initiate an infection in humans (Blaser and Newman, 1982). More recent data support the "independent-action" or "single-organism" hypothesis, which is based partially on a phenomenon observed in laboratory studies – that given proper conditions a single bacterium, virus, or protozoan can reproduce to cause infection (Rubin, 1987). Although each microorganism alone is capable of initiating the infection, infection may still require exposure to more than one organism. More must be ingested because of the relatively small probability that a single microorganism will successfully evade host defenses (Rubin, 1987). This is analogous to another biological phenomenon, that of spermatozoa and fertilization (Rubin, 1987). However, once a single cyst passes the host defenses, it is presumed to be able to colonize and

infect the host. Immunity at the cellular and humoral level may play a critical role in determination of which individuals may develop infection and more severe disease.

The *Giardia* risk assessment model used by Rose et al. (1991b) to assess waterborne risks was based on limited dose-response studies in the 1950s (ICAIR, 1984) which of course were not done with modeling in mind. The *Giardia* risk model is described in terms of the probability of infection (P_i):

$$P_i = 1 - e^{(-rN)}$$

where r is the fraction of microorganisms that are ingested which survive to initiate infection (this is organism specific) and N is the daily exposure assuming consumption of 2 liters of drinking water per day. The parameter for *Giardia* was r=0.0198 (0.009798-0.03582, 95% confidence interval). The assumption of 2 liters per day is conservative, as it is an overestimate of water consumption.

Table VI-3. Dose-Response Assessment for Human and Animal Hosts (Haas et al., 1998)

Infectious Agent	Host	Value of r	Probability of Infection*
G. lamblia	Humans	0.0199	2.0×10^{-2}
G. lamblia	Gerbils	0.0019	2.0×10^{-3}
G. lamblia	Muskrats	0.000004	2.7×10^{-6}
G. lamblia	Humans	0.0698	9.3×10^{-2}
G. muris	Mice	0.56	4.3×10^{-1}

*Probablity of infection for exposure to 1 organism except 9.3×10^{-2} where probability of infection is for exposure to 1 glass of water (an estimated 2 cysts). During one *Giardia* outbreak, a dose-response relationship between glasses of water consumed per day and infection suggested that each glass of water may have contained 2 cysts.

Table VI-3 shows risk estimates for mathematical models developed in a variety of species and hosts. These data indicate that dose-response data for the muskrat, mouse, or gerbil

are not appropriate for use in estimating human risks. For *G. lamblia*, the animal models predicted a lower risk than did the human dose-response data; for *G. muris* however, the risk predicted was greater. Schaefer et al. (1991) found that infective dose levels of *G. lamblia* for the Mongolian gerbil were much higher than those found for human volunteers and for *G. muris* in the murine model. The importance of host specificity and the danger in extrapolation of animal data to humans for infectivity and dose-response modeling is supported by the comparisons in Table VI-3.

D. Exposure assessment

In risk assessment, exposure assessment is an attempt to determine the size and nature of the population exposed and the route, levels and distribution of the microorganisms and the duration of the exposure. The description of exposure includes not only the occurrence of *Giardia* in water but how often the microorganisms are found. Exposure assessment depends on adequate methods for recovery, detection, quantification, sensitivity, specificity, virulence and viability, as well as studies and models addressing transport and fate through the environment. Often the amount of contaminant in the medium associated with the direct exposure (i.e. drinking water or food) is not known and must be estimated from other data bases. Therefore, knowledge is needed about the ecology of these microorganisms, sources, transport and fate including inactivation rates and survival or resistance to environmental factors (temperatures, humidity, sunlight etc) and movement through soil, air and water. Finally, because the current methods for monitoring microorganisms in environmental samples often do not have the necessary sensitivity to detect actual levels in treated drinking water, raw water must be analyzed, and additional data are needed on the inactivation and removal of microorganisms through treatment processes. These data can then be used to estimate levels in treated drinking water.

Water quality monitoring data are needed, and the analytical methods used to obtain these data will greatly influence the estimate of exposure. In the case of *Giardia,* all cysts detected are

assumed to be viable and of the type that infect humans, and this overestimates the risk. However, the risk may also be underestimated due to the inefficiency in recovery and detection of cysts. Studies by Clancy et al. (1994) found that the greatest problem was specificity as opposed to sensitivity and that the level of cysts may be underestimated by almost ten-fold. In order to meet the current safety goal of no more than one *Giardia* infection per 10,000 persons from drinking water exposures, the analytical method must be capable of detecting one cyst in 150,000 L. Because detection of cysts at this level is difficult with the current methodology, source water levels must be monitored and exposures estimated based on presumed water treatment reductions. The many factors that influence the exposure estimate for *Giardia* are described in Chapter III and summarized in Table VI-4. A summary of the water occurrence data is shown in Table VI-5.

Table VI-4. Exposure Factors associated with Assessment of Waterborne *Giardia* Risks

Exposure Factor	Summary Information
Transmission	Fecal-oral, waterborne (drinking and recreational) transmissions appears to be more important than other routes; suggested to be responsible for 60% of all cases.
Environmental sources	Levels found in: Sewage (treated wastewater, secondary effluent) - range of 0.2 to 130 cysts/L & average of 0.88 cysts/L; discharges of 1 MGD would put 3 million cysts into the waters each day) (Rose et al., 1991b). Animals - prevalence in cattle 29%, sheep 38%, pigs 9% and horses 20% (Chap. IV). Storm waters - average 1100/L (Gibson et al., 1998)
Survival potential	Time for 90% inactivation is temperature dependent. Data suggest 1 month is required to achieve 90% reductions at ambient temperatures (Chapter III). Adequate survival model for temperatures not available. Travel time should be delineated from source to exposure.
Regrowth	None
Occurrence in raw water supplies	Levels ranging from 0.005 to 44/L Range of Averages: 0.002 to 2.2 /L (Table III-1)

Resistance to water treatment	Removals by sand filtration ranged from 1.1 to 5.1 log_{10}; slow sand filtration removals ranged from 1.2 to 4 log_{10}; diatomaceous earth removals ranged from 2 to 3 log_{10}; microfiltration removals ranged from 6 to 7 log_{10} (Table VII-1). Coliform bacteria inadequate to evaluate cyst inactivation (45% of all giardiasis waterborne outbreaks where coliform data were reported had no coliform detection). Turbidity and particles appear to be appropriate for evaluating filtration effectiveness but not for occasional spike of contamination or when coagulation not optimized. Inactivation by disinfection can be estimated from *Ct* (Table VII-2). 90% to 99% reductions depending on disinfectant, application and water characteristics, applied throughout in U.S. facilities.
Environmental transport	No transport models available.
Availability of methods	Methods for detection are available (10 to 20% recoveries); variability in assessing levels; no methods for identification of environmental sources (animal versus human), or viability (however 11% viable by animal infectivity) (Chapter VII).

In full-scale seeded experiments, conventional filtration treatment used in drinking water reduced cysts by an average of 3.3 log_{10}. Direct filtration of these organisms showed slightly improved performance reducing and cysts by an average of 3.9 log_{10} (Nieminski & Ongerth, 1995). Removal efficiencies are expressed as either percent removal (e.g., 99%) or more typically in terms of logarithmic (base 10) removal of cysts. For example, a 1 log_{10} removal indicates a 90 percent reduction in densities; 2 log_{10} removal means that 99 percent of cysts are removed; 3 log_{10} removal means that 99.9 percent are removed.

However, monitoring data from actual water plants show that these reductions probably overestimate the effectiveness of the filtration barrier. Data from two surveys of finished drinking water was used to estimate *Giardia* risks: LeChevallier, et al. (1991b) found 17% of treated water samples to be for *Giardia* (cysts ranged from 0.2 to 64 cysts/100L) and LeChevallier and Norton (1995) found *Giardia* in 4.6% of the samples (an average of 2.6 cysts/100 L and a range of 0.98 -9.0 cysts/100 L).

Table VI-5. The Occurrence of *Giardia* in Various Waters

Type of Water	Percent	Range of Cyst Levels (Cysts per Liter)

Untreated Wastewater	100	642-3,375
Activated Sludge Effluent	83	0.14-23
Filtered Secondary Effluent	75	<0.01-0.2
Surface Water	45	<0.02-44
Groundwater	6	0.1-120
Treated Drinking Water	17	0.29-64
Combined Sewer Overflows	100	90-2,830

Source: Rose et al., 1991b; LeChevallier et al., 1991a, b; LeChevallier and Norton, 1995; Hancock et al., 1998; Gibson et al., 1998.

E. Risk Characterization

Risk characterization is an integration of the previously described three steps in order to estimate the magnitude of the public health problem and to understand the variability and uncertainty of the hazard. This encompasses the spectrum of health outcomes, the confidence limits surrounding the dose-response model, the distribution of the occurrence of the microorganism, and the distribution of exposure. The occurrence and exposure can be further delineated by distributions surrounding the method recovery and survival (water treatment reduction) distributions.

The estimates of daily risk of infection associated with the averages and ranges of *Giardia* cysts found in drinking water are shown in Table VI-6. Annual risks of *Giardia* infection from drinking water, including asymptomatic infections, averaged approximately 20 x 10^{-4} (20 infections per 10,000 people annually) and were as high as 250 x 10^{-4} (250 infections per 10,000 people annually. These annual risk estimates are presented as point estimates without confidence limits and do not account for *Giardia* speciation and viability or analytical sensitivity and specificity. Although they have these limitations, they do suggest that the annual risk of infection due to current levels of *Giardia* in treated drinking water may be greater than the recommended annual risk of *Giardia* infection that drinking water systems should attempt to maintain. Point estimates of computed risk are 10 to 100 times the recommended risk level of no more than one *Giardia* infection per 10,000 persons from drinking water exposures.

Table VI-6. Risk Estimates for Waterborne *Giardia* Infection

Exposure	Cysts Levels (Cysts per Liter)	Daily Risk of Infection from 2 L/day
Average (1991 survey)	0.04	1.8×10^{-3}
Range(1991 survey)	0.003 to 0.64	0.1 to 25×10^{-3}
Average(1995 survey)	0.026	1.0×10^{-3}
Range(1995 survey)	0.0098 to 0.09	0.39 to 3.6×10^{-3}

*LeChevallier et al., 1991b; LeChevallier and Norton, 1995.

It is difficult to ascertain from *Giardia* surveillance statistics the accuracy that these risks levels represent because most infections would be asymptomatic and persons that do develop symptoms are not likely to seek medical care and undergo laboratory diagnosis. Estimates of endemic waterborne infection risks may be able to be obtained from epidemiological studies using serological techniques, but such studies have not yet been conducted.

Teunis et al. (1997) recently completed a comprehensive risk assessment of both *Cryptosporidium* and *Giardia* using monte carlo analysis and the distributions rather than single estimates for the following parameters: levels of oocysts and cysts (average <1/1000 L), analytical method recovery effectiveness (<2%), viability of the recovered oocysts (30%) and cysts (15%), removal of protozoa during water treatment based on *Clostridium* spores (2.8 $\log_{10}$), the daily consumption of tap water (0.15 L/day), and dose-response *r* values (*Cryptosporidium* = 0.00467, *Giardia* = 0.01982). The cumulative estimate for an annual risk of waterborne infection ranged from 10^{-5} to 10^{-4} for *Cryptosporidium* or *Giardia* and from 10^{-4} to 10^{-3} from exposure to both organisms. The data used to develop the parameters utilized by Teunis et al. (1997) were specific to the Netherlands with exception of the viability and the dose-response models, and using a similar approach in other geographical areas should result in different annual risks worldwide based the occurrence of protozoa in water, water treatment practices, and water consumption in the area evaluated. For example, in the United States a higher estimate for the

annual risk of waterborne *Giardia* infection is expected because of the higher occurrence and exposure to these protozoa in drinking water. A comparison of the estimated risks of waterborne *Giardia* infection from the Netherlands and the United States computed using the different mathematical models shows that risks in the United States are higher (e.g., 200 to 2500 times greater). This may be due to both higher drinking water exposures and limitations of the model used to compute the risk estimates (e.g., lack of consideration of analytical recoveries and viability in the model).

A marriage of risk assessment methods with epidemiological models that describe the transmission of disease through a population has been suggested as an appropriate approach for examining population risks (Eisenberg et al., 1996). This would take into account factors such as incubation time (time from exposure to infection and illness), immunity (protective as well as impaired) and secondary non-waterborne transmission within the population, in addition to water exposure and the dose-response relationship. However, by considering these factors, the mathematical models can require as many as 13 model parameters with accompanying information about their distributions, and much of this information is currently not well understood. Therefore many assumptions must be made in order to use these models. The simplicity of the Rose et al. (1991b) model make it easy to use, but it also has limitations due to assumptions made. The complexities of the models proposed Eisenberg et al. (1996) may make them more difficult to use, and the more assumptions needed for the additional parameters in the model may make the results more difficult to evaluate. However, these models do attempt to include all of the relevant information that may be needed to estimate waterborne risks and used in combination with a sensitivity analysis should help identify the parameters that may have the greatest effect on the risk estimate. A comparison of waterborne *Giardia* infection risks for the United States computed from each of the models (Rose et al., 1991b; Eisenberg et al., 1996; Teunis et al., 1997) would help risk managers in their interpretation of the risk assessments.

F. Risk Management and Federal Regulations

As technological advancements increase in microbial detection and more knowledge becomes available about risks of multiple microbial exposures in drinking water, directors of municipalities and health officials will be called upon to ensure that the public is adequately protected from epidemic and endemic waterborne disease. Risk assessment is a tool that can be used by health officials and water utility managers to help interpret water quality surveys, assist in defining the adequacy of drinking water treatment, and communicate possible health risks.

Currently, the EPA regulations (U.S. EPA, 1989a, b; 1994a, b, c; 1996) that specifically address *Giardia* in potable supplies are found within the SWTR, ICR, and the proposed Enhanced Surface Water Treatment Rule (ESWTR). The Clean Water Act also regulates point and non-point discharges that may contain fecal material into receiving waters, with fishable and swimmable being the goals, but there are no specific limits for *Giardia* in discharges to recreational areas or to receiving waters that are used as sources for drinking water. Coliform bacteria are used as the indicator of fecal contamination of discharges to source and recreational waters. This approach has a serious limitation in that coliform bacteria are much more susceptible to disinfection than cysts. Effluent discharges may contain low levels of coliform bacteria because they are disinfected, however, levels of *Giardia* may be high. Watershed programs for protection of drinking water sources and emphasis in the future on the safety of recreational sites will mean that occurrence databases from point and non-point discharges will need to be better defined.

The SWTR requires filtration and disinfection of all surface water supplies and groundwater directly impacted by surface water. Because monitoring for waterborne pathogens was deemed impractical, the rule developed a series of treatment requirements for surface and groundwater under the influence of surface water. These requirements specified a minimum removal or inactivation of 3 $\log_{10}$ for *Giardia* and 4 $\log_{10}$ for viruses in water treatment provided; water treatment levels could be increased for poor source water quality. The rule also lowered

the acceptable limit for turbidity in finished drinking water from a monthly average of 1.0 NTU to a level not to exceed 0.5 NTU in 95% of 4-hour measurements. The requirements for meeting these limits went into effect in June 29, 1993. Based on the current risk analysis, most water utilities would be required to provide more than the minimum 3 $\log_{10}$ treatment for *Giardia* specified by the SWTR in order to meet the safety goal of no more than one *Giardia* infection per 10,000 persons from drinking water exposures.

With the development of regulations to limit the levels of disinfectants and disinfectant by-products (D/DBP), the EPA recognized the possibility that efforts to reduce DBP levels could inadvertently increase the risk from microbial agents. To ensure that implementation of the D/DBP Rule (U.S. EPAb), did not increase microbial risk, the EPA considered it necessary to examine the health and economic implications of various approaches to DBP regulation, to compare microbial risk from *Giardia* infection to cancer risk for several DBP control scenarios, and to review the adequacy of the existing SWTR.

The data generated by the ICR will be used to help formulate the final draft of the ESWTR and it is likely that the final rule will be subject to many modifications. Water treatment plant performance may require greater reliability and removal of *Giardia*, and alternative treatments such as membrane filtration and use of ozone may be considered. Cost and benefit assessments will be required, and risk assessments will be used to evaluate the benefit side of producing better drinking water quality. The rule will likely be developed to protect against waterborne risks of *Cryptosporidium.* Cryptosporidiosis has a high mortality in immunocompromised persons and the infection is not treatable. Also, *Cryptosporidium* is smaller than *Giardia* and may be more difficult to removal by conventional filtration and much more resistant to disinfection. However, *Giardia* cyst levels in sewage and wastewaters are very high, the waterborne exposure to *Giardia* is likely to be greater, and the dose-response model suggests it is more infectious. Thus, it is important to consider waterborne risk assessments for both *Giardia* and *Cryptosporidium.*

VII. ANALYSIS AND TREATMENT OF *GIARDIA*

In order to make informed decisions about drinking water treatment and regulations for *Giardia*, reliable data are required on the occurrence and distribution of the organism in the environment and in human and animal populations. There is also a need to assure that drinking water treatment regimes for removing or inactivating cysts are adequate. Interpreting available data on the occurrence, distribution and treatment effectiveness for *Giardia* in water supplies requires an understanding of the capabilities and the limitations of the methods used to collect the data. Classical cultural techniques for microorganisms, such as are used for bacteria and many of the enteric viruses, are not applicable to detecting, identifying and enumerating *Giardia*. This Chapter will review methods for detecting cysts in water, clinical diagnosis procedures, and effectiveness of drinking water treatment.

A. Analysis in Water

1. Detection and Identification Methods

The previous *Giardia* Criteria Document (ICAIR, 1984) reviewed an available method for detecting cysts in water. Significant steps in the method included sample collection and sample processing. Sample collection was accomplished by filtering large volumes of water (about 100 gallons or 380 L were recommended as the sample size) through microporous filters constructed of tough fibers (Orlon or polypropylene). Sample processing involved procedures (filter extraction, sedimentation, centrifugation, flotation) to recover the cysts from the filter and separate them from interfering debris and other organisms. The final step in sample processing was detection and identification using microscopic examination of iodine stained material. The development of a cultural technique was not considered likely, and although a viability determination might be accomplished by animal feeding, this could only be done by laboratories equipped with the necessary isolation and animal handling facilities.

Jakubowski (1984) reported the results of a *Giardia* method workshop convened to address issues such as a "reference method", cyst identification, viability and suitable applications for the methods. The reference method (also known as the zinc sulfate flotation method or the zinc sulfate/Lugol's iodine method) was essentially as described in ICAIR (1984), but many of the steps within the method had not been verified under controlled laboratory conditions and were based on experience and professional judgement of the participants. For *Giardia* cyst identification, the consensus was that "Suspect organisms should possess the proper size and shape and at least two internal characteristics (nuclei, axostylar rods, median bodies)." A minority opinion was that only one internal characteristic should be required if the organism was of the correct size and shape, but the majority felt that this could result in misidentification. Suspect objects that met all requirements for identification except the possession of two internal characteristics should be reported as *"Giardia* cyst-like". It was recommended that the examination of sample concentrates and identification of cysts be conducted by competent, well-trained individuals who have a demonstrated proficiency for diagnosing intestinal protozoa.

For determining cyst viability, the workshop participants noted that animal testing was problematical, excystation testing of cysts detected in water samples was questionable, and dye staining methods (at that time, eosin exclusion) did not correlate with in vitro excystation. Participants considered the current methods for viability suitable only for use in outbreak investigations or special research applications but not for routine monitoring or surveillance. In addition, a consensus recommendation was that viability determinations were not necessary for water samples collected in outbreak investigations where epidemiological data implicated the drinking water. Since *G. lamblia* cysts were only obtainable at the time from human stools, workshop participants encouraged the identification of a suitable laboratory animal host and encouraged the investigation of immunological methods for detection and identification.

Sauch (1985) developed an indirect immunofluorescence assay for detecting and identifying *Giardia* cysts in water samples. The assay used a polyclonal primary antibody

developed in rabbits against whole *G. lamblia* cysts obtained from an asymptomatic donor. The secondary antibody was goat anti-rabbit IgG conjugated to fluorescein isothiocyanate (FITC). The assay was evaluated against a variety of human and non-human (beaver, dog, mice, vole, muskrat, gerbil) source cysts and bright apple green fluorescence was produced by cysts from all of these sources when the specimens were illuminated with UV light. Sauch (1985) indicated that the assay allowed rapid location of cysts even in samples that contained significant quantities of other microorganisms and debris. When fluorescing cysts of the appropriate size and shape (presumptive *Giardia*) were located, phase-contrast microscopy was used to examine them for internal characteristics in order to confirm their identity.

In applying the assay to water samples which were collected in outbreak investigations and processed using the reference method (except that Percoll-sucrose was used for flotation instead of just sucrose or zinc sulfate), Sauch (1985) was able to detect and confirm *Giardia* cysts in 58% (14/24) of raw water samples and in 27% (6/22) of distribution system samples. She pointed out that failure to confirm an object did not mean it was not *Giardia* since internal morphology often cannot be discerned in some known *Giardia* cysts even though they fluoresce brightly. In the water samples she examined, 13% (3/24) of the raw water and 40% (9/22) of the distribution system samples contained objects that fluoresced and were the right size and shape but could not be confirmed by internal morphology. As recommended by the consensus reference method, these water samples were reported to contain cyst-like objects.

In developing a method to recover *Cryptosporidium* from water, Musial et al. (1987) based the sample collection procedure on the *Giardia* reference method procedure using 1 µm nominal porosity polypropylene filter cartridges. Although they did not evaluate their method with *Giardia* cysts, they did find that using 0.1% Tween 80 as the eluent instead of just water resulted in higher recoveries of *Cryptosporidium* oocysts. They also found that adding Tween 80 and sodium dodecyl sulfate detergents to the centrifuged pellet from the filter elution and

sonicating resulted in the highest recovery of oocysts in sediment-containing preparations. Sonication did not help to recover particles from the filter material itself.

Rose et al. (1988b) evaluated a method similar to that of Musial et al. (1987) for detecting both *Giardia* and *Cryptosporidium* in water. They tested six gradient solutions for recovering *Giardia* cysts. Although recoveries ranged from a low of 40% for zinc sulfate (specific gravity, 1.18) to 77% for Percoll-sucrose (specific gravity, 1.09) there was no statistically significant difference in these results. They noted that potassium citrate (specific gravity, 1.16) and 4/5 Sheather's (specific gravity 1.24) gave cleaner preparations when used with environmental samples. They did not find any statistically significant increase in recoveries with the use of sonication. For detection in a direct fluorescence assay, they used 5 µm porosity cellulose nitrate membrane filters and monoclonal antibodies conjugated to FITC.

Stibbs et al. (1988) investigated mouse monoclonal antibodies developed against cysts of *G. muris, G. simoni* and *G. lamblia*. The *G. muris* antibodies reacted with homologous cysts and with rat source cysts but not with cysts from beaver, dog, human, muskrat or vole. The *G. simoni* antibodies reacted only with rat and cow cysts. The *G. lamblia* antibodies reacted with all human, beaver, dog and rat source cysts but not with *G. muris* or cysts from muskrat or vole. The authors suggested that systematic differences occur in cyst surface antigens and that it might be possible to develop a monoclonal antibody-based typing scheme for strain and animal source identification.

Januschka et al. (1988) tested the reactivity of antibodies produced against cysts of *G. lamblia* and *G. muris* with cysts of *Spironucleus muris*. *Spironucleus* sp. share morphological characteristics including axonemes, nuclei and peritrophic space with *Giardia* sp. And they are both in the same family, Hexamitadae. Januschka et al. (1988) found that all of the antibodies tested reacted with both *Giardia* and *Spironucleus* cysts, indicating that these antibodies were not genus specific. *Spironucleus* cysts are somewhat smaller than *Giardia* cysts, averaging about 5 x

8 µm, but they share morphological characteristics and are widely distributed in animals, and the authors advised caution in confusing *Spironucleus* and *Giardia* cysts when examining water samples.

Payment et al. (1989) reported studies on development of a method to concentrate *G. lamblia* cysts, *Legionella pneumophila*, *Clostridium perfringens*, human enteric viruses and coliphages from large volumes of water using a single filtration. They used electronegative fiberglass cartridge filters of 3 µm and 1 µm nominal porosity in series to collect conditioned water (pH 3.5, 0.001 M aluminum chloride) at a flow rate of 10-40 L/min. Filters were eluted by backflushing with 1.5% beef extract, pH 9.75 containing 0.5% Tween 80. After adjusting the pH to 7.2 with 1.2 N HCl, the eluate was centrifuged and the pellet was further processed for *Giardia* by re-suspending a portion on a discontinuous sucrose density gradient and centrifuging. The interfaces were collected and examined by phase microscopy or immunofluorescence. In seeded studies with about 10,000 cysts, the efficiency of retention by the filters was >99%, the recovery after elution was 71% and the final recovery after flotation and centrifugation was 52%.

As the immunofluorescence technique for cyst detection became more widely used, the number and variety of antibodies available increased. Rose et al. (1989) evaluated four antibodies for detecting *Giardia* in environmental samples. In preliminary trials, one of the antibodies that was developed against trophozoites would not react with *G. lamblia* or *G. muris* and was not studied further. The remaining three antibodies had been developed against cysts and were of the IgG type; two used an indirect fluorescence procedure and one used a direct procedure, and two were monoclonal antibodies and one was polyclonal. The monoclonal direct antibody did not react with *G. muris* cysts but the other two did, and the polyclonal antibody detected more *G. muris* cysts than did the monoclonal. *G. lamblia* cyst counts with one of the monoclonal antibodies and with the polyclonal antibody were evaluated in three storage media (deionized water, 2.5% potassium dichromate and 3.7% formaldehyde). Cyst counts were 29% higher when they were stained with the polyclonal antibody. There were no significant

differences in cyst counts among the three storage media. After 20 to 22 weeks storage at 4°C, there was no significant association between cyst counts and either the formaldehyde or potassium dichromate storage media. However, cyst counts were reduced 67% in the deionized water and those cysts remaining had diminished fluorescence and distorted shape.

Laboratory studies revealed differences among the antibodies with regard to species identification and number of cysts detected, but when applied to environmental wastewater samples, there were no significant differences in cyst counts even though one of the monoclonals consistently produced higher maximum counts. The precision of determining cyst counts on membrane filters was evaluated with four replicates from three cyst preparations stained with a monoclonal antibody. The coefficient of variation ranged from 7.89 to 11.62 and averaged 9.22 at cyst densities between 24 and 70/filter. Rose et al. (1989) also tested the reaction of antibodies with cysts exposed for 20 minutes at room temperature to sodium hypochlorite at concentrations of 5 to 20,000 mg/L. At exposures up to 50 mg/L, the counts remained stable with all three antibodies. With the monoclonal antibodies, *Giardia* cyst counts were decreased by 35% to 57% at exposures of 500 mg/L, and no cysts were detected at exposures higher than 5,000 mg/L. With the polyclonal antibody, cyst counts remained stable at sodium hypochlorite concentrations up to 500 mg/L and decreased 66% to 83% at higher exposures. Sauch and Berman (1991) also investigated the effect of chlorine, at levels that would be expected to encompass the range used in drinking waters, on the morphology and fluorescence of cysts. They used exposure temperatures of 5° and 15°C, residual chlorine levels from 1 to 11 mg/L, and an exposure time of 48 hours. The majority of the cysts lost their internal morphology but were still detectable by immunofluorescence. They concluded that if chlorinated (>1 mg/L) water samples are to be examined for cysts, the majority of them will lose morphology necessary for identification if they are collected, transported, and stored for more than four hours without neutralizing the chlorine.

Concerning species identification as previously indicated, differences were observed in specificity of the antibodies for *G. lamblia* and *G. muris*. However, Rose et al. (1989) concluded

that until questions of species definition in *Giardia* are resolved "...it is unlikely that any single antibody will identify cysts in water which only pose a health risk to humans." The authors indicated that while immunofluorescence has increased the ability to detect cysts in water, underestimation of densities in samples is a significant problem. This is due to a combination of factors including the length of time the organisms have been in the environment, the type of antibody used and the amount of debris in the samples. They also expressed concern about the lack of information on the viability of detected organisms.

LeChevallier et al. (1990) compared the zinc sulfate flotation/Lugol's iodine reference method to the immunofluorescence (IFA)/Percoll-sucrose method for detecting *Giardia* cysts. They used a monoclonal antibody (Meridian Diagnostics, Cincinnati, OH) in an indirect assay adapted from Sauch (1985). When tap water concentrates were spiked with 550 to 5,145 *Giardia* cysts, recovery by the zinc sulfate method averaged 5.9% (range = 1.6% to 13.5%). With the IFA method, recovery averaged 74.1% (range = 37.1% to 92.7%). In comparing both methods for the detection of *Giardia* cysts in natural raw water samples from rivers, they found on average about 12 times (range = 1.5 to >40) more cysts with the IFA method. They concluded that the Meridian antibodies were convenient and reliable regents for the water utility laboratory and that the IFA/Percoll-sucrose method is more efficient in detecting parasites in water than the zinc sulfate flotation method.

Abbaszadegan et al. (1991) developed and evaluated a gene probe method for detecting *Giardia* cysts in water samples. Their method used a 265-base pair (bp) cDNA probe from the small subunit rRNA of *G. lamblia*. They evaluated 6 protocols for extracting nucleic acids from cysts and found they could detect approximately 1 to 5 cysts (determined by dilution) by a dot blot hybridization assay when they used glass beads and proteinase K to disrupt the cysts. Specificity testing against 15 other microorganisms including parasites, bacteria and yeast produced negative results with the probe. The assay was specific at the genus level since it detected *Giardia* from mice, birds, beaver and humans, although with different sensitivities. The

authors suggested that specificity at the genus level might be more desirable for evaluating drinking water treatment efficiency, and that species level specificity would be desirable for evaluating health hazards. A limited number of water and sewage samples were examined by the IFA method and by the gene probe method. Positive results were obtained by both methods in two samples and in a third, only by the IFA method. An advantage of the IFA method is that it quantitatively detects cysts. Gene probe methods can be made semi-quantitative by using quantal assays, but intact cysts might not be detected.

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Mahbubani et al. (1991) reported the development of PCR amplification techniques for detecting *Giardia* cysts. DNA was released from cysts by heating and a 171-bp region of the giardin gene was amplified by PCR. The giardin gene was selected as a target because this is a potentially unique protein found in the ventral disk of *Giardia* (Crossley et al., 1985). The amplified product was detected with gel electrophoresis, ethidium bromide staining and Southern hybridization of radio-labeled gene probes. The specificity was tested against 19 *Giardia* isolates (including human beaver, muskrat, cat, mouse and bird strains) and 24 isolates of other microorganisms (including protozoa, bacteria, algae and yeast). A 171-bp PCR product was only produced by the *Giardia* isolates. Using single cysts recovered with a micromanipulator, these investigators demonstrated that the assay was sensitive enough to detect one cyst.

Mahbubani et al. (1992) also developed a PCR and gene probe method for differentiating *G. duodenalis* from other *Giardia* species. Human, muskrat, beaver, bird, cat and mouse source cysts all produced a 171-bp PCR amplification product with primers GGL639-658 and GGR789-809. Confirmation of specificity for all *Giardia* species tested and none of the non-*Giardia* species was obtained through Southern blot analysis with a radio-labeled probe (GGP751-756). When cysts were spiked into 100 mL river water and potable water samples at densities of less than 1/mL, they were detectable by PCR. They were also detectable by PCR when spiked in 4-liter river water samples when they were present at equal to or greater than 0.25 cysts/mL. PCR

did not detect cysts in concentrates from 400 L river samples even when spiked at cyst densities of 10^5/sample.

In 1992, the ASTM published a proposed test method for detecting and enumerating *Giardia* cysts and *Cryptosporidium* oocysts in low turbidity ground and surface waters. Low turbidity was defined as equal to or less than 1 nephelometric turbidity unit (NTU). The method was designated 'proposed' because the precision and accuracy of the method had not yet been determined in accordance with ASTM requirements. The method involves collecting a recommended minimum sample volume of 100 gal (380 L) by filtration, at flow rates up to 4 L/min, through a 1 m nominal porosity polypropylene cartridge microporous filter. The filter is eluted with a detergent solution which is then centrifuged to recover particulates. The pelleted material is subjected to a flotation purification procedure using Percoll-sucrose solution (specific gravity 1.1). The purified material is applied to a membrane filter in a concentration that will result in a depth not exceeding a monolayer. After staining the material with indirect fluorescent antibody assay reagents, the membrane is mounted on a glass slide and subsequently examined using a combination of epifluorescence and phase or differential interference contrast microscopy. Results are reported as presumptive or confirmed cysts or oocysts/100 L using specific criteria for immunofluorescence, size, shape and internal morphological characteristics to define the categories. Known interferences with the detection and identification of cysts and oocysts were listed as including turbidity due to inorganic and organic material, autofluorescing or nonspecific fluorescing organisms and debris, chemical compounds and freezing samples, eluates, or concentrates. The method will not identify the species of protozoa detected or the species of the host of origin nor will it provide information on the viability or infectivity of the cysts. The proposed method was intended for use with raw and treated drinking waters, and it was suggested the method may be useful in identifying contamination sources and in evaluating water treatment effectiveness.

Turbidity continued to pose challenging problems for investigators attempting to determine cyst levels in many source water supplies, primarily rivers. Bifulco and Schaefer (1993) explored the application of an immunomagnetic procedure for selectively recovering cysts from water sample concentrates. They developed an indirect antibody-magnetite method using a mouse IgG anti-*Giardia* antibody as the primary antibody. The secondary labeling reagent was an anti-mouse IgG antibody-coated colloidal magnetite particle with an average size of 40 nm. They chose the small size magnetite particles to prevent potential interference with microscopic visualization of the cysts. Cysts labeled with the magnetite reagent were concentrated using high-gradient magnetic separation. The mean recovery of cysts from water samples with various turbidities (70 to 6,400 NTU) was 82%; 90% of cysts seeded into buffer were recovered. Cyst recoveries were highest at turbidities below 600 NTU. Bifulco and Schaefer (1993) felt this method had the potential for being linked with IFA detection methods since the cysts were already coated with anti-*Giardia* antibody.

Erlandsen et al. (1994) proposed a molecular approach using fluorochrome-rDNA probes to differentiate species and detect *Giardia* by *in situ* hybridization. Carboxymethylindocyamine dyes were conjugated to oligomeric probes (17-22 mer) to the 16S-like rRNA of *G. lamblia, G. muris,* and *G. ardeae*. Alternatively, the oligomeric probes were labeled by incorporating a fluorescent marker (e.g., fluorescein) to the 5' end of the oligomer. They were able to specifically identify *G. lamblia* and *G. muris* cysts in the same sample using specific rDNA probes each conjugated to a fluorochrome producing a different color when examined with a confocal microscope having a dual krypton-argon laser. Also, using a combination of the fluorochrome-rDNA conjugates and antibodies to the cyst wall, they could identify *G. lamblia* cysts in fecal samples and in samples from a sewage lagoon. The *G. lamblia*-specific probe did not hybridize to *G. duodenalis*-type organisms from the parakeet, great blue heron, and the vole. This latter finding led Erlandsen et al. (1994) to strongly suggest that *Giardia* from these animals is most likely different from *G. lamblia* and that *G. duodenalis* does not have any continued value as a species name for *Giardia*.

Clancy et al. (1994) indicated that the proposed ASTM (1992) IFA method was considered to be the method of choice for detecting *Giardia* and *Cryptosporidium* for a planned nationwide monitoring effort. They conducted a blinded survey of 12 laboratories performing protozoa analyses with environmental samples, i.e., the laboratories were not informed of the study so they should not have given special treatment to the samples. Known private, government and university laboratories providing this type of analysis were contacted; sampling equipment was obtained and evaluated; filters were spiked with known quantities of sediment, cysts, oocysts, or algae; the filters were returned to the laboratories for analysis, and the results from each laboratory were evaluated. Filters were spiked with about 387 formalin-preserved *G. lamblia* cysts or with about 500 *Oocystis minuta* algal cells. Of the 12 laboratories, 11 submitted reports, but only seven laboratories found *Giardia* cysts. One laboratory reported cysts present without providing quantitative data; the remaining laboratories reported recoveries ranging from less than 1% to 22%. Only three laboratories discussed the differences between presumptive and confirmed cysts; a fourth mentioned that internal features were not found in the cysts. Of the reports received for water samples containing algae, two laboratories incorrectly identified these as *Giardia* cysts. Clancy et al. (1994) concluded that not all laboratories were strictly following the ASTM analytical method, even though they said they were and that the majority of laboratories needed to improve in one or more of the following areas: client response, quality of sampling equipment, sampling directions, turnaround time for result reporting, quality of data and report format.

In the 18th edition supplement to *Standard Methods for the Examination of Water and Wastewater* (APHA-AWWA-WEF, 1994) and in the 19th edition (APHA-AWWA-WEF, 1995) the previously described zinc sulfate flotation method was replaced with a method basically the same as the ASTM (1992) IFA method. Significant differences in the method included reporting requirements and a limit for the turbidity of water to be sampled. A limit of 1 NTU or less is required by ASTM; no turbidity limit is in *Standard Methods*. Reporting of results differed from

the ASTM method in that the presumptive/confirmed categories were replaced with total count/count with internal structure categories. In addition, the *Standard Methods* version was published as a proposed method. It was noted that the method had been developed by a consensus panel and not standardized and that modifications might be needed depending upon local water quality conditions, equipment availability and analyst experience. The difficulty of interpreting positive and negative findings was also discussed.

In 1994, the EPA proposed the Information Collection Rule (ICR) which required water systems serving populations of 10,000 people and using either surface water or groundwater under the influence of surface water to monitor for *Giardia* and *Cryptosporidium* (U.S. EPA, 1994a). Monitoring was to be conducted by the ASTM method as modified by expert workshops and performance evaluation studies. The principal differences between the ASTM and ICR methods included: no turbidity limitation for the ICR monitoring; use of differential interference contrast (DIC) or Hoffman modulation optics for visualizing internal structures in cysts rather than phase contrast microscopy as specified by ASTM; use of goat serum as a blocking agent to minimize nonspecific immunofluorescence in the ICR method; stringent positive and negative quality control requirements in the ICR monitoring rather than the less stringent, non-mandatory ASTM recommendations; mandatory requirements for the use of filters to collect primary water samples and immunofluorescence reagents; and differences in porosity of the assay membrane. There are also differences in the reporting of results; ICR uses the total count terminology whereas ASTM uses the presumptive/confirmed terminology. The final ICR method was published along with the regulation (U.S. EPA, 1996).

In reporting the development and application of flow cytometric methods for detecting cysts and oocysts in water, Vesey et al. (1994) cited deficiencies in the IFA method including the inefficiency of the method, requirement for skilled analysts, tediousness of the examination due to the frequent necessity for spending hours examining a single sample, and interferences from non-target particles in the sample. The investigators compared direct microscopy of concentrates

to flow cytometry for detecting cysts and oocysts in sewage effluents, reservoir and river water samples, and dam water and tank water samples. The sewage effluents were concentrated by centrifugation; the reservoir and river water samples (100 L) were concentrated by tangential flow filtration, and the dam water and tank water samples (10 L) were concentrated by calcium carbonate flocculation. A direct antibody technique was used for the microscopy and the flow cytometry. Two flow cytometers were used, either a Coulter EPICS V or a Coulter Elite (Coulter Corp., Hialeah, FL). Preparations from environmental samples were stained with monoclonal antibody in suspension and analyzed directly without any washing steps. All particles demonstrating the fluorescence and light scatter characteristics of cysts and oocysts were examined by epifluorescence microscopy after flow cytometry cell sorting (FCCS) onto glass microscope slides. In six river and reservoir samples spiked with 77 *Giardia* cysts, recoveries by direct microscopy ranged from 80% to 91%; by FCCS/microscopy recoveries ranged from 92% to 115% with each of the six values being higher than that obtained by direct microscopy. In seven unspiked samples from sewage effluents, dam water and tank water, cyst counts by direct microscopy ranged from 1 to 420 (mean = 75.7) whereas by FCCS/microscopy the range was 4 to 611 (mean = 121.0) with each sample being significantly higher by the latter technique. Vesey et al. (1994) concluded that the FCCS technique simplified and improved analysis of water samples for cysts and oocysts with high recoveries and without operator fatigue problems.

Ho et al. (1995) developed a technique for recovering *Giardia* cysts from river waters and from other environmental waters that depended upon concentrating the samples by the flocculation-Percoll/sucrose gradient method followed by immunofluorescence staining with FITC labeled anti-*Giardia* monoclonal antibodies. The procedure had a cyst recovery rate of 61% compared to zero recovery for a membrane filter method and 4% for a method using polypropylene filter cartridges.

LeChevallier et al. (1995) conducted tests to evaluate the sources of losses of *Giardia* cysts and *Cryptosporidium* oocysts in the IFA method. For *Cryptosporidium*, major losses were

found to occur in the centrifugation and clarification (flotation) steps. For *Giardia* major losses occurred only in the centrifugation step. With a swinging-bucket rotor, *Giardia* cyst losses decreased from 25% to 11% when the relative centrifugal force (RCF) increased from 1,040 to 6,700 x *g*. With a fixed-angle rotor, cyst loss decreased from 40% to 24% when the RCF increased from 800 to 5,000 x *g*.

Nieminski et al. (1995) evaluated two different approaches to the IFA method; one was the ASTM procedure for low-turbidity water samples, and the other procedure involved sampling less water (40 L) by filtration through a large diameter (142- or 293-mm) membrane filter (2.0 m porosity), followed by concentration on a Percoll-Percoll step-gradient. The goal was to find the best method for evaluating the effectiveness of water treatment processes. In their laboratory, the ASTM method resulted in a 12% average recovery of cysts while the membrane filter method recovered 49%from water samples spiked with 1,000 cysts/16 L. Evaluation of spiked river water, filtered water and flocculation basin water having turbidities of 5 NTU, 0.5 NTU and 20 NTU, respectively, indicated that both methods more efficiently recovered *Giardia* cysts compared to *Cryptosporidium* oocysts and that the sampling step resulted in the highest loss of both protozoa. The membrane method was less time-consuming and cheaper but did not allow determining presumptive and confirmed cysts and oocysts. Therefore, the potential for falsely identifying a cyst or oocyst would be increased when cross-reacting algae were present. The investigators suggested investigation of a hybrid method that combined the most efficient steps of both methods.

Rodgers et al. (1995) studied the potential for algal interference when using the IFA method. They tested 54 algal species for cross-reactivity with the antibodies used in the assay. Twenty-four species exhibited some degree of fluorescence with the ASTM antibody reagents while two species, *Navicula minima* and *Synechococcus elongatus*, produced a bright apple green fluorescence. Adding goat serum to the assay mixture blocked the fluorescence of most nontarget organisms while decreasing background fluorescence on the membrane. The

fluorescence of *Giardia* cysts and *Cryptosporidium* oocysts and the identification of internal structures was not compromised by the addition of goat serum.

Fredericksen et al. (1995) compared the IFA membrane technique with a proposed well-slide modification. The results indicated that the well-slide method was superior to the membrane technique. Danielson et al. (1995) found FCCS to be more accurate and sensitive than the IFA method, however, attempts to determine viability using dyes and flow cytometry were inconclusive. They used a dual color dye system, FITC (green) and phycoerythrin (red), to label cysts and indicated that this enhanced the sorting of the cysts.

Bielec et al. (1996) recently proposed a modified procedure for the collection and recovery of *Giardia* cysts from diverse water sources. The method involves the use of a compact, battery-operated, portable field filtration apparatus. Cyst recoveries from seeded samples ranged from 60% to 80% (mean = 66.1%). The design of the sampling unit also permits spiking to allow the introduction of *Giardia* cysts during the filtration step which allows developing estimates of recovery during the sampling procedure. Charles et al. (1995) described an alternative bench-scale spiking procedure for use in the laboratory.

Shepherd and Wyn-Jones (1996) studied cartridge filtration, membrane filtration and flocculation for simultaneous recovery of cysts and oocysts. They evaluated different procedures to optimize the density of the organisms. In both tap water and river water, cyst recovery was highest when organism suspensions were centrifuged at 5,000 x *g* as compared to 1,500 x *g*. Also, an antibody staining technique where the cysts were stained in suspension was found superior to a technique that used direct staining on a multiwell slide. The calcium carbonate flocculation method gave the highest recoveries in seeded river water and tap water samples for both cysts and oocysts. Examination of a variety of 142-mm membrane types and porosities indicated that a cellulose nitrate filter with a porosity of 3.0 µm produced the best cyst recoveries.

LeChevallier et al. (1997) presented results on the occurrence of cysts and oocysts in open reservoirs and discussed various methodological considerations. They noted that a 100% variation in recovery rates with the ICR method is not unexpected because of the number of steps where losses of organisms can occur. Before examining water sample concentrates in their laboratory, analysts examined positive control fecal samples comparing characteristics of environmental isolates were compared with the control organisms. As a result, they indicated that the definition of *Giardia* and *Cryptosporidium* is primarily an operational definition where the identification of environmental isolates depends on agreement of the morphological characteristics with those in positive controls. As a quality control measure, all analysts in their laboratory were required to count the number of cysts and oocysts in a positive control on a weekly basis. Variation of more than 30% from the mean of all counts was an action level that triggered a review of microscopic procedures and a discussion of method technical details.

Examination of the results from the reservoir sampling revealed that, although sample volumes of the influent and effluent waters were the same (1,579 L), the equivalent volumes for each type of sample varied by about 250% (75 L for influent samples and 32 L for effluent samples). The pellet volumes were not very different but the big difference in amount examined was probably due to a difference in the nature of the material in the pellet. The effluent samples had higher amounts of algal cells that could interfere with cyst and oocyst detection. While there is controversy over whether internal morphological characteristics can be affected by sampling or analytical procedures, the authors indicated that they have never observed this with spiked samples examined in their laboratory. In reviewing recovery efficiencies, LeChevallier et al. (1997) cited the EPA data from performance evaluation (PE) studies among 10 laboratories that indicated a 30% recovery efficiency for *Giardia* cysts with a 77% coefficient of variation; recovery in their laboratory was similar with 34.5% recovery and 73% coefficient of variation. LeChevallier et al. (1997) also indicated that the EPA studies involved spiked filters whereas recovery efficiencies in their laboratory included the sample collection step. Jakubowski et al. (1996) summarized two EPA round-robin PE studies that indicated 25 to 44% mean recovery

efficiencies for *Giardia* with 58 to 93% coefficients of variation. The EPA also conducted a field spiking/sampling study during 1995 at seven water utilities (ARCTECH 1996). These field spiking and laboratory analyses for *Giardia* cysts showed recovery rates of 36.7% for raw waters, and 26.4% for finished waters, similar to those in the PE studies.

Stewart et al. (1997) reported the development of a device to collect "first flush" water in rugged terrain during storm events. Accessing these sites can be problematical during storm events and the devices were constructed to function unattended. They consist of a 5 L polyethylene contained fitted with an adjustable PVC valve. There are four 1/8-in openings in the valve through which water enters. The samplers were installed on the upstream side of existing creek weir structures. Each weir had a gauge that adjusted the sampler valve to a predetermined flow. Samplers were retrieved within 24 hours of a storm event and returned to the laboratory for processing. These samplers proved superior to 100 L filter samples or to 4 L grab samples in terms of ease of use, positivity of samples, and levels of cysts detected.

Hoffman et al. (1997) compared IFA and FCCS for detecting cysts and oocysts in 262 fresh or archived (preserved and stored) samples. The FCCS procedure was not performed concurrently with the IFA procedure but may have been carried out as long as 16 months after the IFA results were obtained. With this set of samples they found little difference in detection of cysts with either method: 116/262 samples were positive by IFA and 114/262 were positive by FCCS. They did find a difference when they examined the results taking archive storage time into consideration. For 132 samples that were analyzed by FCCS within 5 months of the IFA analysis, 70 were positive for cysts with 43 detected by IFA and 55 detected by FCCS. This difference was significant ($p=0.04$) but there was no statistically significant difference between the methods with samples that had been stored for 1, 10 or 16 months. There was also no difference in results for *Giardia* cysts with 26 samples purchased from a private laboratory and analyzed simultaneously by IFA and FCCS.

Ionas et al. (1997) developed a PCR method using random amplification of polymorphic DNA that could differentiate *G. muris* from *G. intestinalis*. They developed primers specific for *G. muris* and suggested that they could be used in combination with primers for *G. intestinalis* to determine if drinking water is safe for human consumption.

Rochelle et al. (1997) evaluated four pairs of PCR primers and probes previously reported for *Giardia* while investigating the effects of primer annealing temperature, magnesium concentration, specificity, sensitivity and PCR additives on the assays. The primers were directed to heat-shock protein (HSP), giardin, and small-subunit rRNA genes, and reported sensitivities ranged from <1 to one cyst. Only one primer pair was specific for *G. lamblia* and the other three were specific at the genus level. After preliminary evaluation and due to inefficient amplification, one primer pair (the one that targeted the small-subunit rRNA) was eliminated from further consideration. A test of multiplex PCR for *Giardia* and *Cryptosporidium* resulted in a sensitivity level of one cyst and one oocyst in purified preparations and 50 cysts and oocysts in seeded water samples. The authors concluded that PCR has the potential to be effective for detecting cysts and oocysts in water but that primer evaluation and optimization was necessary for obtaining suitable sensitivity and specificity. The *Cryptosporidium* primers with the greatest sensitivity and specificity were not compatible with any of the *Giardia* primers for multiplex PCR. An ideal combination of sensitivity, specificity and compatibility with multiplex PCR was not demonstrated by any of the available primers.

Kaucner and Stinear (1998) described a reverse transcription-PCR (RT-PCR) assay for direct analysis of primary sample concentrates from large volume water samples to simultaneously detect viable oocysts and cysts. The assay incorporated an internal positive control (IPC) to assess the efficiency of mRNA isolation and the potential for RT-PCR inhibition. The method used the fiberglass filter sampling and elution procedure recommended by Payment et al. (1989), selective capture of mRNA with oligo $(dT)_{25}$ magnetic beads, and detection of viable cysts by RT-PCR. The IPC hybridized to the magnetic beads and allowed

monitoring the process from mRNA capture through RT-PCR on a per sample basis. The intent was to develop a multiplex PCR but sensitivity of detection decreased 10-fold in this system, so separate PCRs were used for *Giardia* and *Cryptosporidium*. Also, these investigators initially tried to use the HSP primers reported by Abbaszadegan et al. (1997) but found this RT-PCR to be unreliable. Instead, they used the giardin mRNA primers indicated by Mahbubani et al. (1991).

Kaucner and Stinear (1998) found positive signals in spiked 100 µL packed pellet concentrates from river and creek samples at all cyst levels tested (2 to 340 cysts/100 µL). They compared RT-PCR and an IFA technique for detecting cysts in 29 environmental water samples ranging in volume from 20 to 1,500 L. Cysts were detected in 24% of the samples by IFA and in 69% of the samples by RT-PCR. Cysts were detected in treated sewage effluents, river water and creek water but not in treated drinking water. The authors considered the combination of a large volume sampling method with RT-PCR to be a significant advance in protozoan pathogen monitoring. They did indicate that a limitation of the method is that it is not quantitative. In addition, the giardin primer set used was genus specific, not species specific.

2. Determination of Viability

Although one of the workshop recommendations reported by Jakubowski (1984) indicated that viability determinations were not necessary in certain outbreak investigations, the viability or infectivity of *Giardia* cysts found in drinking water at other times is important to determining their public health significance and the need for regulations.

Hoff et al. (1985) compared animal infectivity and excystation for quantitatively determining the viability of *G. muris* cysts. With their mouse model, they found that 1 to 15 cysts constituted an infectious dose. For cysts exposed to chlorine, they concluded that in vitro

excystation was an adequate indicator of *G. muris* infectivity and that it could be used to study disinfectant effect on viability.

Schupp and Erlandsen (1987a) evaluated a method for determining viability based on staining of *Giardia* cysts with the fluorogenic dyes fluorescein diacetate (FDA) and PI. Cysts stained with FDA fluoresce green and those stained with PI fluoresce red when subjected to light of the appropriate wavelength by epifluorescence microscopy. These investigators determined that *G. muris* cysts that took up the PI would not cause infections in mice when they were inoculated with either 5,000 or 50,000 cysts. However, cysts stained with FDA readily produced infections in mice. They concluded that FDA-positive cysts are viable and that PI-positive cysts are nonviable as determined by animal infectivity.

Schupp and Erlandsen (1987b) also compared FDA/PI staining to DIC, phase, or bright field microscopy for determining viability. Using *G. muris* cysts, they determined that cysts incorporating FDA were morphologically identical and had (1) a clearly defined cyst wall, (2) a distinct space between the cyst wall and the cytoplasm and (3) polar flagella. When examined under DIC, the cytoplasm had a hyaline appearance that made detection of the internal characteristics difficult. In PI stained cysts, the internal characteristics could be seen and a space was not observed between the cyst wall and the cytoplasm. They indicated that further testing was necessary in order to determine the reliability of these criteria for *G. lamblia*.

Schaefer (1988) reviewed methods used to determine *Giardia* cyst viability and concluded that excystation was reliable and reproducible for *G. muris* cysts but not for *G. lamblia* cysts. For both species of *Giardia*, the factors found to promote excystation included low pH, the presence of carbon dioxide, a temperature of about 37°C and a final neutralization at pH 7.0. *G. muris* cysts did not require a maturation period before producing maximum excystation, but *G. lamblia* did. Schaefer (1988) indicated that the fluorescent dye staining and morphological criteria methods for determining viability were new and might require validation

but in a subsequent review, Schaefer (1990) concluded that fluorogenic dyes correlated well with excystation in *G. muris* but that data on *G. duodenalis* were inconclusive. Schaefer (1988) also noted disadvantages to animal infectivity for determining viability. Only some *Giardia* isolates from humans will infect gerbils, and those that infect gerbils may not produce cysts requiring necropsy to verify infection. Infectivity is dose dependent, and the failure to produce infection with a given inoculum of cysts does not mean that the cysts were not alive or not infective for humans. Other disadvantages include high cost and the need for maintaining approved laboratory animal facilities.

Sauch et al. (1991) evaluated PI with *G. lamblia* cysts for compatibility with the previously developed IFA test (Sauch, 1985). They found that the PI stain was compatible with this method which uses a fluorescein label for detecting cysts in water samples. They also studied PI as an indicator of viability in *G. muris* cysts exposed to heat, chlorine, chloramine and a quaternary ammonium compound. There was a correlation between PI and excystation for cysts inactivated with either heat or the quaternary ammonium compound. In both cases, the percentage of cysts stained with PI tended to lag the percentage of intact cysts. This could indicate that the cysts were injured or damaged to the point where they could not excyst while the cyst wall was still intact and prevented entry of the PI. There was no correlation between excystation and PI staining with chlorine or chloramine-exposed cysts. For the chlorine results, the authors speculated that this strong oxidizing agent may have masked or destroyed the sites within the organism needed for the PI to be detected. With chloramine, the authors suggested that the lack of correlation may have been due to decreased permeability in the cyst wall from exposure to this disinfectant. The authors stated that any tests proposed for determining the viability of *Giardia* cysts should be evaluated with cysts inactivated by agents relevant to water supply disinfection.

Mahbubani et al. (1991) developed a PCR test for distinguishing live from dead cysts using giardin mRNA as the target. Cysts of *G. muris* and *G. lamblia* were killed by freezing,

heating and chloramine exposure. The PCR test was compared to excystation and animal infectivity. *G. muris* cysts that had been killed by any of the three methods did not excyst and did not produce infections in animals. A positive PCR signal for the 171-bp diagnostic region was obtained indicating that the target DNA was preserved in dead cysts. However, when the giardin mRNA was used as the target, a positive signal was produced in cysts killed by heat or monochloramine but was not was produced in cysts after freezing. *G. lamblia* cysts produced similar results. Live cysts that were induced to initiate excystation produced greater amounts of RNA that could be measured spectrophotometrically, thus allowing dead cysts to be distinguished from live cysts. There was no increase in RNA when cysts killed by any of the three methods were induced to excyst.

Linquist (1995) described the use of a dual fluorochrome method for the detection of *Giardia lamblia* cysts and the determination of their viability. The use of the IFA method involves light microscopy but requires switching the optical components from fluorescence to DIC in order to verify the internal morphological characteristics for confirmation of the *Giardia* cysts. Linquist (1995) reported on the use of a dye combination, FDA and Texas Red ® hydrazide (TRH), with the FDA being taken up by viable cysts, and the TRH being actively excluded. When each of these two dyes were used, together or separately, in combination with a fluorescent antibody specific for *G. lamblia* cysts, the dual labeling permitted identification of the internal morphological structures and good observations without the need to convert to DIC optics. However, the procedure was accomplished with confocal microscopy, a technology currently found in research laboratories and not in laboratories performing routine analyses.

As part of a study on the prevalence and characterization of *Giardia* cysts isolated from Canadian drinking waters, Wallis et al. (1996) compared PI exclusion and gerbil infectivity as indicators of viability. When tested by dye exclusion, the average viability of 167 drinking water and sewage samples that were found positive for cysts was 24.6% (n=127) and 38.9% (n=40), respectively. Gerbils were inoculated with cysts from 33 of these samples and 8 (24%) infections

were produced. The authors did not note any clear relationship between dose and infection. Also, based on failure to infect gerbils with many samples containing viable cysts, they stated that the similarity of viabilities by PI exclusion and gerbil infectivity was coincidental. There are factors other than the number and viability of inoculated cysts that determine the probability of infection with some isolates. They found that 11/194 (57%) isolates from water samples infected gerbils even when no *Giardia* cysts were detected in the sample. Wallis et al. (1996) concluded that cysts isolated from water and sewage have viabilities much less than 100% and the gerbil model is not always accurate because some strains do not infect gerbils. They also indicated that the PI technique is much easier to use and works well with the indirect IFA when PI is added during the last 5 minutes of incubation with the primary antibody. Satisfactory results were not obtained with direct antibody kits or with using PI after the FITC conjugate has been added.

Abbaszadegan et al. (1997) developed a PCR viability assay for *Giardia* that was based on heat-shock protein (HSP). They used an *hsp-70*-like gene that coded for HSP and was specific to *G. lamblia* to develop the assay. The basis of the assay is that a cell cannot produce HSP mRNA when exposed to elevated temperatures unless it is viable. They also developed a presence/absence test for *Giardia* based on amplification of total DNA or RNA from lysed cysts. The PCR viability procedure involved heating cyst-containing samples to 42°C for 15 min; freeze-thaw the preparation through 5 cycles; use magnetic beads to isolate the mRNA; heat the sample to denature proteins, then go through PCR amplification and detection of a 163-bp product. *Giardia* DNA or the corresponding RNA from lysed cysts was detectable by the HSP primer set. The test was sensitive at levels of one cyst but is not quantitative and does not determine viability. Using the same primers, an amplification product was detectable in heat-shock induced cyst at a sensitivity level of 10 cysts. The product was not produced in live cysts that had not been heat induced, nor in dead cysts killed by heating. The authors suggested that PCR was an attractive method for detecting and determining the viability of *Giardia* cysts, and that the method had advantages of speed, cost, sensitivity and specificity.

As indicated previously, Kaucner and Stinear (1998) used RT-PCR to detect viable *Giardia* cysts in environmental water samples. The primer set they used was that of Mahbubani et al. (1991) which detected giardin mRNA. Mahbubani et al. (1991) had used this primer set to differentiate live from dead cysts by inducing excystation in the cysts and determining whether an increase had occurred in the mRNA signal after induction. Mahbubani et al. (1991) found that heat killed cysts and cysts killed by monochloramine produced a positive signal for giardin mRNA, so just detecting it would not indicate viability. However, in contrast, Kaucner and Stinear (1998) found that heat inactivated cysts did not produce or maintain mRNA. They suggested that the discrepancy might be related to experimental differences such as the time between heat treatment and mRNA extraction. They did not address the issue of a positive signal produced by monochloramine treated cysts.

B. Detection in Biological Samples

The following methods commonly used for diagnosis of giardiasis were summarized in the previous *Giardia* Criteria Document (ICAIR, 1984): (1) direct microscopic examination of fecal smears for cysts or trophozoites, (2) identification of motile trophozoites in specimens from the upper intestines, (3) intestinal biopsies or (4) gastrointestinal radiology using barium. However, it was pointed out that duodenal aspirations, biopsies and radiological techniques were not suitable for routine screening of human or animal populations and there were no clinically accepted serological methods for diagnosis of giardiasis.

Wolfe (1984) indicated that stool examination should be the initial diagnostic procedure of choice and that it should be performed by an experienced technician who was using appropriate collection and laboratory procedures. When formed stools are examined, the cyst form is more likely to be present in fresh, unpreserved specimens. Loose or watery stools should be immediately examined in a wet smear of a just-passed specimen or preserved using one of several formalin-containing preservatives or commercially available kits. If parasites are

numerous, direct smears may be adequate for examination but concentration of the sample using formol-ether or zinc sulfate flotation may be necessary for light infections. Lugol's iodine may be used to stain wet smear preparations.

Riggs et al. (1983) were the first to report development of an immunofluorescence assay for *Giardia* cysts in fecal specimens. They developed high titer sera to *G. lamblia* cysts and conjugated that to fluorescein isothiocyanate. There was no cross-reactivity when tested against the following protozoan parasites: *Iodamoeba bütschlii, Dientamoeba fragilis, Entamoeba histolytica, Entamoeba coli* and *Endolimax nana.* In stool samples, the conjugate did cross-react with cysts of *Chilomastix mesnili* which fluoresced as intensely as the *Giardia* cysts. However, the authors indicated that the smaller *Chilomastix* cysts could be easily differentiated.

Ungar et al. (1984) developed an ELISA specifically for the detection of *G. lamblia* in fecal samples. ELISA methods are rapid, accurate, inexpensive, and require a lower degree of technical training than microscopical analyses of fixed slides. The assay was capable of detecting between 37 to 375 cultured trophozoites and 12.5 to 125 cysts purified from human stool. The sensitivity of the test was found to be 92%; ELISA produced 36 positives in 39 specimens known to be positive for *Giardia* by direct examination of formalin-fixed stool specimens or by biopsy. A high specificity (98%) was also found; only 3 of 128 stool specimens from patients without demonstrable giardiasis were positive.

Erlandsen et al. (1990c) compared two methods for determining the prevalence of *Giardia* in beaver and muskrat populations, the detection of cysts in fecal samples of kill-trapped animals and examination of mucosal scrapings from live-trapped animals. Examining the intestinal contents resulted in significantly higher prevalence rates of infection in these animals. Based on detection of cysts in fecal specimens, the prevalence of infection in muskrats and beavers was 36.6% and 9.2%, respectively. By examining intestinal contents for trophozoites, the prevalence of infection in muskrats and beavers increased to 95.9% and 13.7%, respectively.

The results indicated the superiority of the mucosal scraping technique for determining the prevalence of infection in these animal populations. Erlandsen et al. (1990d) also studied the recovery of cysts from animal tissues and fecal samples subjected to cycles of freezing and thawing. They found that cysts might not be detected in specimens of this type if only bright field microscopy was used for the examination. However, the cysts were detectable by immunofluorescence even though the cyst walls had been distorted.

Wolfe (1992) again reviewed procedures for diagnosis of giardiasis and described a number of possible problems that could prevent identification of *Giardia* in clinical specimens. These included: (1) medication administration -- could cause organism distortion, low numbers of organisms or mask their presence; (2) diagnostic procedures – may cause organism distortion; (3) radiographic examination -- barium may cause organism distortion and mask their presence; (4) intermittent shedding -- numbers of organisms in stool fluctuate widely; (5) specimen collection -- trophozoites may disintegrate if fixative not used; (6) laboratory techniques -- using concentration techniques and preparing permanent stained fecal smears are mandatory; (7) specimen examination -- requires trained personnel, and (8) interpretation of results -- failure to obtain additional specimen types when needed, e.g., duodenal biopsies. He also summarized the sensitivity and specificity of 11 rapid detection immunoassays for *Giardia* including enzyme immunoassays (EIA), counterimmunoelectrophoresis, and indirect immunofluorescence. One of these was directed at detecting cysts; the others all targeted specific antigens. The reported sensitivity of these assays ranged from 88% to 98%; specificity ranged from 87% to 100%.

Xiao and Herd (1993) developed and evaluated a quantitative direct fluorescent antibody (DFA) assay. For the purpose of uniformity in this section, DFA will be used in place of FA, DIF or other descriptors that the authors might have used in their published articles to indicate an immunofluorescence assay employing a direct antibody. Their test had a theoretical sensitivity of 100 *Giardia* cysts/g of feces and they compared recovery rates with this test to those obtained with sucrose gradient and zinc sulfate flotation methods. Their procedure used the commercially

available MERIFLUOR® *Cryptosporidium/Giardia* antibody kit (Meridian Diagnostics, Cincinnati, OH). In calf feces seeded with 1,000, 10,000 and 100,000 cysts/g the recovery rates were 76.4%, 96.9% and 89.6%, respectively. In contrast, the recovery rates using sucrose gradient flotation were 20.5%, 51.2% and 42.9%, respectively. Zinc sulfate flotation detected 36.4% of infections when the cyst level was equal to or less than 1,000 cysts/g.

The DFA assay was compared to routine stains (saline and iodine-stained wet preparations; chlorazol black E and Kinyoun acid-fast stained permanent smears) for detecting *Giardia* in 2,696 fresh human fecal specimens examined in a hospital clinical laboratory (Alles et al., 1995). These investigators also used the MERIFLUOR® antibody kit. The DFA assay produced a significantly increased detection rate; the sensitivity by routine examination was 66 % compared to 99% DFA. A limitation of the DFA assay is the requirement of an epifluorescence microscope that many hospital clinical laboratories might not have.

Stazzone et al. (1996) used MERIFLUOR® antibody staining to retrospectively evaluate human stool specimens processed by a laboratory in Egypt. The laboratory was reporting abnormally low identification rates for *Giardia* and *Cryptosporidium* using conventional dye staining methods (trichrome for *Giardia* cysts). Antibody staining was shown to be almost 3 times more sensitive for detecting cysts than conventional trichrome staining. There was no significant difference in the results whether fresh or frozen stools (stored at equal to or less than 70°C) were used. This latter finding led the authors to suggest that the immunofluorescence method could be used for retrospective quality control on frozen specimens when fresh specimens were not available.

Karanis et al. (1996b) used a different antibody kit, *Giardia*-CEL® (Cellabs P.L., Sydney, Australia), to compare phase contrast microscopy and the DFA assay for detecting *Giardia* cysts in cattle and wild rodent feces. Of 40 cattle fecal specimens examined, 31(78%) were positive by DFA and only 17 (55%) were positive by phase contrast microscopy. All of the detected cysts

were identified as belonging to the *G. duodenalis* group. *Giardia* was found in 103/216 (48%) fecal specimens collected from wild rodents. In 97 (94%)of the samples, DFA was positive; phase microscopy detected 57 (55%) positives. Both methods were positive for 51samples. Only 19/103 wild rodent specimens found positive contained cysts belonging to the *G. duodenalis* group. The authors concluded that, while DFA was more sensitive for detecting cysts, phase microscopy was required to differentiate at the species level.

Hassan et al. (1995) detected *G. lamblia* antigens in stool samples before and after patient treatment with a double antibody sandwich ELISA method. The sensitivity of the assay was 98% with a high specificity. The authors found a direct relationship between the levels of antigens in stool samples and the numbers of cysts detected.

Dixon et al. (1997) compared conventional microscopy, immunofluorescence microscopy and flow cytometry for detecting *Giardia* cysts in beaver fecal samples. They examined 94 formalin preserved beaver fecal specimens that had been concentrated by sucrose flotation. For the conventional microscopy, one drop of floated suspension was placed on a slide and examined by scanning at 400X magnification. Immunofluorescence microscopy was performed using 20 µL of floated fecal suspension air dried on a microscope slide, stained with *Giardia*-CEL® antibody (Cellabs, distributed by Wellmark Diagnostics Ltd., Guelph, Ontario) and scanned at 400X magnification. Flow cytometry used 200 µL of floated fecal suspension stained with the same antibody and processed in a FACSCAN (Becton Dickinson, Mississauga, Ontario) flow cytometer. Of the 94 specimens, 7 were positive by conventional microscopy, 9 by immunofluorescence microscopy and 14 by flow cytometry. Those found positive by flow cytometry were verified by cell sorting and examination under immunofluorescence microscopy. The authors concluded that flow cytometry was more rapid than the other techniques and allowed for screening a larger number of samples within a given time period. They suggested using this technique for prevalence studies in animals and for screening human clinical specimens during outbreak situations.

Mank et al. (1997) compared microscopy and EIA for examining human stools for *Giardia*. Their results indicated that EIA techniques may be about as sensitive using a single stool sample as microscopy is when using two sequential stool samples.

Garcia and Shimizu (1997) evaluated commercial diagnostic kits for detecting *Giardia*: two were DFA kits and 5 were EIA kits. One of the DFA kits (MERIFLUOR®) was used as the reference method. The kits were tested against 100 formalin preserved stool specimens that were found positive using the reference method and an additional 50 specimens that were negative with that method. The other DFA kit was the TechLab *Giardia/Crypto* IF kit (TechLab, Blacksburg, VA). The sensitivity and specificity of the TechLab kit was 100% when compared to the reference method. However, the authors indicated that the fluorescence intensity of the TechLab reagents was one level lower than that of the reference method. In the evaluation of the EIA test, 92/100 specimens were positive by all 5 kits, and with the negative samples, all 5 kits correctly identified 50/50. All kits performed within expected levels as stated in the manufacturer's documentation for sensitivity and specificity. The authors suggested that the decision on which kit to use was up to each laboratory and would depend on factors other than sensitivity and specificity, such as cost or ease of use.

Aldeen et al. (1998) also examined EIA kits for detecting *Giardia* in fecal specimens. In addition to sensitivity and specificity, they compared other factors such as ease of use, cost and processing time. They evaluated nine kits but only four of these were still available at the time the article was published. They found sensitivities ranging from 88% to 100% and specificities of 99% to 100%. Total hands-on time to run one specimen ranged from 1 to 2.25 minutes. The cost per test generally ranged between five and six dollars. The authors suggested replacing three exams for ova and parasites with a single EIA when the likely clinical diagnosis is giardiasis.

Ortega and Adam (1997) prepared a state-of-the-art clinical article in which they concluded that the use of serodiagnosis (i.e., using patient serum samples instead of stool samples) to detect giardiasis was not useful because the available methods cannot distinguish between present and previous infections. Adam (1991) had earlier concluded that serologic testing was useful for epidemiological studies but not for diagnosis of individual patients because of sensitivity and specificity problems. Ortega and Adam (1997) also indicated that fecal specimens have inhibitors that reduce the sensitivity of PCR for diagnosing giardiasis. Marshall et al. (1997), in a review of waterborne protozoan pathogens, suggested that nucleic acid-based detection methods are challenging due to difficulty in lysing the cysts. However, they indicated that the large amount of DNA and the presence of inhibitory substances in clinical specimens were more important. There has been only one published report on the application of PCR to human samples and that investigator experienced some false-negative and false-positive results when comparing PCR to a microscopic reference method (Weiss, 1995).

Rosales-Borjas et al. (1998) studied the secretory immune response during natural *Giardia* infections in humans by examining saliva samples. For antigen, they used the membrane-rich protein fraction of cultured trophozoites. They were able to demonstrate a secretory IgA response in the saliva of infected patients that was not present in healthy individuals. They indicated their results were from only 24 patients, but if substantiated, they could have significance for the isolation of important protective or diagnostic *G. lamblia* antigens.

C. Water Treatment Practices

Information from laboratory, pilot plant, and full scale treatment plant studies demonstrate that *Giardia* cysts can be effectively removed or inactivated by commonly used water filtration technologies and disinfectants. A combination of water filtration and disinfection operated under optimum conditions can protect against waterborne transmission of giardiasis.

In this document, removal and inactivation efficiencies are expressed as a percent removal or inactivation (e.g., 99%) or in terms of the logarithmic (base 10) removal or inactivation of *Giardia* cysts. For example, a 1 $\log_{10}$ removal or inactivation indicates a 90 percent reduction in cyst levels; a 2 $\log_{10}$ removal means that 99 percent of cysts are removed; 3 $\log_{10}$ removal means that 99.9 percent are removed. In some instances, removal is shown as a greater than value which is calculated when no cysts are detected in the filtered water; the calculations are based on *Giardia* cyst analytical detection limits for the methodology used..

1. Filtration

The filtration technologies most frequently used to remove microbial contaminants and particles that cause turbidity from water sources are: conventional filtration, direct filtration, slow-sand filtration, diatomaceous earth (DE) filtration, and membrane filtration. Conventional filtration refers to the use of rapid-rate filters that are composed of granular material, either all sand or dual/mixed media (e.g., anthracite-sand, anthracite-sand-garnet, activated carbon-sand) preceded by chemical coagulation, flocculation, and sedimentation; filtration is followed by disinfection. An important process in conventional filtration is flocculation which allows the suspended particles in the water to form into a larger mass. Sedimentation allows the heavier floc-particle masses to settle before the water is filtered. In some instances, flotation rather than sedimentation is employed to reduce suspended particles. *Giardia* cysts and other pathogens may become enmeshed in the floc-particle masses. Direct or in-line filtration with dual or mixed media is often used for water sources with low turbidity and uniform water quality. This type filtration does not employ the sedimentation or flotation process. After the addition of coagulant or filter-aid chemical(s), sufficient time is allowed for mixing and coagulation, usually in the water pipes, before the water is filtered. Higher filtration rates are often used in direct filtration.

During the filtration process, particles in suspension enter a deep bed of the granular material, which contains a random network of interconnected pores. The water flow is laminar in these deep-bed granular filters, and in such a flow and pore structure, *Giardia* cysts will not be significantly removed by straining. Rather, physico-chemical interactions govern the attachment of suspended particle masses and cysts to the surface of sand and other filter media. Chemical coagulation by aluminum or ferric salts, or the addition of polyelectrolytes, ensures effective filtration of very small size particles and microorganisms including *Giardia* cysts. Coagulant chemicals hydrolyze and form hydroxide precipitates in water, providing positive potentials which interact with and attract particles and cysts in the water. These particle masses are more easily attracted to filter media and removed principally due to their larger size and electro-charge. Most rapid-granular filters operate by gravity flow but are sometimes operated under pressure. Pressure and gravity filtration employ the same process, and the operating principles are identical. Coagulation is necessary for effective removal of *Giardia* cysts by either gravity or pressure sand filters.

Al-Ani et al. (1986) demonstrated the importance of coagulation and optimum dosage of coagulant chemicals in pilot- and full-scale treatment plants operated in both conventional and direct filtration modes. Effective coagulation adequate to reduce turbidity from 0.5 to 0.1 NTU was capable of removing 95% to 99.9% of *G. lamblia* cysts. Filtration efficiency was similar for conventional and direct filtration. However, when no chemical coagulation was used, removal of *G. lamblia* cysts was very poor, ranging from 0 to 50%. Similar poor removals were obtained when ineffective coagulants or improper dosages were used.

Filtration by rapid granular filter media is not effective for 100% removal of cysts. Not all *Giardia* cysts will be removed and some cysts that are removed may become dislodged. This must be recognized and steps should be taken to optimize the filtration process and to monitor each filter to detect changes in water quality and it effectiveness. An increase in turbidity or particles in the filter effluent may indicate filter breakthrough (i.e., the ineffective removal of

cysts or the release of previously removed cysts). This may occur towards the end of a filter run when the filter efficiency is poor, during the restarting of a filter after it has been cleaned, when filtration flow rates are increased, when coagulation dosages are inadequate, or when the water source becomes more contaminated. Filter breakthrough should be closely monitored. Ongerth (1990) found that major deficiencies in the operation of three small water plants with either conventional filtration, direct filtration, or DE filtration caused *Giardia* cyst removals to range from about 40% to 99%. These deficiencies included poor optimization of chemical addition and on-off filter cycles without backwashing. For the sand filters, filtering to waste after backwashing was recommended, as the initial period of filtration immediately after backwashing indicated the potential for passage of cysts.

Bellamy et al. (1993) reviewed various treatment plant performance factors that may affect the removal of *Giardia* cysts. Especially important are: adequate rapid mix-coagulation with appropriate chemical coagulants; appropriate flocculation times and sufficient volumes and baffling within the sedimentation tanks; adequate depths for filtration and use of multiple media filters (e.g., sand and anthracite). Where the turbidity of raw water is low and direct filtration is used, it was recommended that source waters be monitored for episodes of higher turbidity and contamination with *Giardia* cysts. To maintain their high flow rates and removal effectiveness, filters must be frequently backwashed to remove material that has been retained in the filters. Because clean filters are less efficient immediately after the backwash process, operational procedures should include provisions to slowly increase the flow rates to the filters and there should be sufficient time for ripening of the filter prior to being placed back online with continuous measurement of turbidity. For a short period of time after being restarted, the filtered water should be discharged to waste.

Sand in slow-sand filters has a much smaller effective size than that used in conventional or direct filtration and the filtration rates are greatly reduced. Removal is accomplished by

physical-chemical and biological mechanisms within the top layers of sand (Weber-Shirk and Dick, 1997). Straining of particles is the dominant mechanism within the filter cakes, and inter-particle attraction is responsible for particle removal in the filter bed. Biologically mediated particle removal was observed for particles smaller than 2 μm. Generally, no pre-treatment is used with slow-sand filters, but some may be preceded by coagulation, sedimentation, or roughing filters which remove large size particles that may clog the filter necessitating more frequent cleaning. DE filtration is also used for the direct treatment of surface waters with relatively low levels of turbidity. Water is filtered through a precoat cake of DE filter media that has been deposited on a support membrane; additional body feed of DE is continuously added to the raw water to maintain the filter cake permeability. Pressure-driven membrane filtration processes used for municipal water treatment are categorized by the effective size of the membranes (i.e., what is the largest particle, colloid, or molecule that can pass through the membrane). The four categories of membranes, in order of increasing removal effectiveness of micron-size contaminants are: microfiltration, ultrafiltration, nanofiltration, reverse osmosis. Membrane systems may also require pretreatment to remove material that can clog or foul the membranes.

The effectiveness of *Giardia* cyst removal by various filter technologies has been evaluated using a challenge of *G. muris* or *lamblia* cysts or beads of a similar size in laboratory-, pilot-, and full-scale test conditions. Field studies of *Giardia* removal have also been conducted to evaluate the effectiveness of operating filtration plants. The ability of particle counters to accurately size *Giardia* cysts has been investigated, and particle counting methods have been used to quantify removal efficiencies of water filters. Results have shown that particle removal can be indicative of microorganism removal, although particle counters may tend to undersize the organisms (O'Shaughnessy et al., 1997).

In order for the results of pilot-scale studies to be meaningful, pilot plants must be properly designed to reflect the conditions of a specific treatment process(es) in an actual

treatment plant, and the influence of various operational factors must be understood and controlled to the extent possible (McTigue and MacPhee, 1997). When results of pilot- and full scale systems are compared, similar cyst removals are often not found. Even the results of pilot-scale studies may differ when conducted in areas where water quality differs. Many factors may influence the removal effectiveness observed in these studies, and the interpretation of their results depends on a thorough evaluation of the study design, operating conditions, and water quality characteristics. Important considerations include: the levels of cysts used in seeding studies and how the cysts are added to the system; cyst source, age and preparation; water quality characteristics, especially water temperature, pH, turbidity; water system demand or flow rates; coagulant chemicals and dosages, and methods of sample collection and analysis of cyst levels in raw and filtered water. To determine removal effectiveness, the level of cysts in the raw water is compared with the level in filtered water. In some studies, the level of cysts in raw water was calculated based on the number of seeded cysts added to the raw water while in other studies the level of cysts is measured from samples of raw water collected after cysts are added. Calculated versus analytically measured cyst levels in raw water often provide a different measure of seeded cysts that will be then compared with an analytically measured level in the filtered water. Different removal effectiveness can also be due to the operational dynamics and hydraulics between pilot- and full-scale plants and among pilot plants in different areas. *Giardia* removal efficiencies from pilot- and full-scale filtration studies are summarized in Table VII-1. Brief descriptions are provided for the studies reported in the Table.

Table VII-1. Summary of Removal Effectiveness of Various Filtration Processes

Type of Filtration	Experimental Design	Removal $\log_{10}$	References
Conventional	Pilot-scale	3.4-5.1	Patania et al., 1995
Conventional	Pilot-scale	3.4	Nieminski & Ongerth, 1995
Conventional	Pilot-scale	1.1->3	Logsdon et al., 1985
Conventional	Full-scale	3.3	Nieminski & Ongerth, 1995
Conventional	Field	2-2.5	LeChevallier et al., 1991b
Conventional	Field	>2.2->2.8	LeChevallier & Norton, 1992
Conventional	Field	>5	Payment and Franco, 1993
Conventional	Field	1.5-1.7	States et al. 1995, 1997
Conventional	Field	1.5	Kelley et al., 1995
Direct	Pilot-scale	3.1-3.6	Ongerth & Pecoraro, 1995
Direct	Pilot-scale	1.5-4.8	Patania et al., 1995
Direct	Pilot-scale	3.3	Nieminski& Ongerth, 1995
Direct	Full-Scale	3.9	Nieminski & Ongerth, 1995
Package plant	Full-scale	<1->3	Horn et al., 1988
Slow Sand	Pilot-scale	>3-4	Bellamy et al., 1985; Jakubowski, 1990
Slow Sand	Pilot-scale	>3-4	Jakubowski, 1990
Slow Sand	Pilot-scale	2.8->4	Schuler et al., 1991
Slow Sand	Field	1-2	Fogel et al., 1993
DE	Pilot-scale	>2->3	Logsdon et al., 1981; Jakubowski, 1990
DE	Pilot-scale	>3	Lange et al., 1986
DE	Pilot-scale	>3	Schuler et al., 1991

Microfiltration	Pilot-scale	6-7	Jacangelo et al., 1995

a. Conventional and Direct Filtration

Logsdon et al. (1985) conducted pilot-scale studies to evaluate sedimentation efficiency and removal of *G. muris* cysts with various types of media (granular activated carbon, sand, coarse anthracite, and dual-media). In waters with turbidities of 27 to 32 NTU, sedimentation of alum-coagulated water resulted in 65% to 83% removals of *Giardia* cysts; in waters with turbidities of 7.5 to 15 NTU, sedimentation of water coagulated with alum and a slightly anionic polymer resulted in 79% to 93% removals of *Giardia* cysts. In evaluations that compared removals among different filter media, coarse anthracite did not perform as well as the other types of filter media when only alum was used; its performance was improved by use of the polymer. Logsdon et al. (1985) noted that cyst levels were higher during the initial phase of the filter run. This emphasizes the need to provide for filter ripening after a backwashed filter is placed back into service, and for a short period of time after being restarted, the filtered water should be discharged to waste. These studies showed that 3 $\log_{10}$ of *Giardia* cysts can be removed and decreased removals are associated with increased turbidity indicating filter breakthrough.

Patania et al. (1995) conducted pilot-scale studies of conventional filtration of waters with turbidities between 0.2 and 13 NTU and *Giardia* cyst levels between 10 and 200/L. With treatment optimized for turbidity removal, *Giardia* cyst removal ranged from 3.4 to 5.1 $\log_{10}$ during stable filter operation. Although the median turbidity and particle removals were only 1.4 and 2 $\log_{10}$, respectively, the median *Giardia* cyst removal was 4.2 $\log_{10}$. A filter effluent turbidity of 0.1 NTU or less resulted in the most effective *Giardia* cyst removal. *Giardia* cyst removal was 0.2 to 1.8 $\log_{10}$ higher during conventional treatment, which included sedimentation, compared to direct filtration. *Giardia* cyst removal was reduced by up to 1 $\log_{10}$

when the filter effluent turbidity increased from 0.1 to 0.3 NTU. *Giardia* cyst removal was generally 0.4 to 0.5 $logs_{10}$ lower during filter maturation or ripening after it had been backwashed.

Nieminski and Ongerth (1995) evaluated both direct and conventional filtration in pilot- and full-scale water treatment plants in studies using *G. lamblia* cysts that had been inactivated by heat and formalin. The pilot plant was operated with a filter loading rate of 5.75 gpm/sq. ft. (gallons per minute per square foot of filter surface area) at 0.5 gpm. The full-scale plant was operated with a filter loading rate of 4.8 gpm/sq. ft. at 600 gpm. Turbidities in the source water for the full-scale plant varied from 2.5 NTU to 11 NTU during the spring and were as high as 28 NTU during August. The source water for the pilot-scale plant typically had turbidities of 4 NTU. In the pilot-scale studies, *G. lamblia* cyst removals averaged 3.4 and 3.3 log_{10} for conventional and direct filtration, respectively, when filtered water turbidities were between 0.1-0.2 NTU. When the full-scale plant achieved similar filtered water turbidities, *G. lamblia* cyst removals averaged 3.3 log_{10} for conventional filtration and 3.9 log_{10} for direct filtration. Differences in the performance of direct filtration and conventional treatment in the full-scale plant were attributed primarily to different source water quality. These studies also showed that removals of cyst-sized particles and turbidity are useful indicators of cyst removal effectiveness.

Ongerth and Pecoraro (1995) evaluated the removal of *Giardia* cysts obtained from infected animal fecal material in a very low turbidity source water (0.33 to 0.58 NTU). The 1-gpm pilot plant used multimedia filters operated in direct filtration at a loading rate of 5 gpm/sq. ft.; alum coagulation was used, and a filter maturation or ripening period was allowed. With optimal coagulation, 3.1 to 3.6-log_{10} removals of *Giardia* cysts were obtained. In one test run, where coagulation was intentionally suboptimal, cyst removal was only 1.3 log_{10} even though the filtered water turbidity was less than 0.5 NTU This emphasizes the importance of maintaining optimum coagulation for effective *Giardia* cyst removal.

A packaged dual-stage filtration system for small water systems was evaluated by Horn et al (1988). In two Colorado river waters, *G. lamblia* removals ranged from <1 to 2 $\log_{10}$ in a water with turbidity of 4 NTU to >3 $\log_{10}$ in a water with turbidity of <1 NTU.

Surveys of municipal water supplies have also provided data to evaluate the effectiveness of water filtration. Since not all water treatment plants are operated in the most efficient manner, surveys of *Giardia* occurrence in water samples from full-scale water treatment should provide more realistic information about actual removal than pilot-plant studies where operation is highly controlled. Rose et al. (1991a) analyzed 257 water samples collected from water sources and potable water from 17 states in the United States. *Giardia* cysts were found in 16% of the surface waters at an average level of 3 cysts per 100 L. Although *Cryptosporidium* oocysts were found in 4 (14%) of 28 treated drinking water samples from systems using conventional and direct filtration, no *Giardia* cysts were detected in any of these samples. Although these results suggest that the facilities sampled were effectively removing *Giardia* cysts, the levels of cysts in source waters were relatively low, and sampling was limited. Chauret et al. (1995) reported data for the removal of *Giardia* cysts from the raw drinking water in the water treatment plants in Ottawa, Ontario. No cysts were detected in any treated water samples from the treatment plants, even though cysts were detected in 83% of the raw water samples at the plant intakes.

LeChevallier and Norton (1992) evaluated *Giardia* occurrence in source and filtered waters at three locations with high (1.8-120 NTU), moderate (3.5-75 NTU), and low (0.4-25 NTU) turbidity. The geometric mean number of cysts detected in raw water at each location was 2.9, 5.8, and 9.1 cysts per L, respectively. The detection of *Giardia* in treated water samples depended primarily on the number of cysts in the raw water, and reported removals at each location were >2.3, >2.8, and >2.2 $\log_{10}$, respectively. In a more extensive monitoring of 347 surface water samples collected between 1988 and 1993, LeChevallier and Norton (1995) found *Giardia* present in 54% of the samples collected. In a survey conducted during 1991 to 1993, water samples were collected from 72 surface water plants in 15 states and 2 Canadian provinces

(LeChevallier and Norton, 1995). *Giardia* cysts were detected in filtered water on 12 occasions (4.6% of 262 samples). When the *Giardia* cysts were detected, there was an average of 2.6 cysts/100 L (range = 0.98 to 9.0 cysts/100 L). Earlier, LeChevallier et al. (1991a, b) had conducted a survey of the occurrence of *Giardia* cysts and evaluated removal efficiencies for *Giardia* in 66 surface water treatment plants in 14 States and 1 Canadian province. Most of these water systems achieved between 2 and 2.5 $\log_{10}$ removals for *Giardia*. *Giardia* cysts were detected in 17% of the 83 filtered water effluents that were sampled. The geometric mean for the positive samples was 4.45 cysts per 100 L with a range of 0.29 to 64 cysts per 100 L. *Giardia* was frequently found in filtered water at facilities with poor quality source waters. For treatment plants with *Giardia*-positive samples, an average of 2.14 $\log_{10}$ removal was found. For *Giardia*-negative plants, >2.45 $\log_{10}$ removals were calculated based on the analytical detection limits of the methodology. Water treatment plants studied used sand, granular activated carbon (GAC), dual media or mixed media filtration systems. Effluent samples from dual media and mixed media filtration plants were more likely to be negative for *Giardia* cysts, while effluent samples from the GAC and rapid sand filter type plants were more likely to be positive (LeChevallier et al., 1991b).

In two conventional water filtration plants, Kelley et al. (1995) observed a 1.5 $\log_{10}$ mean removal of *Giardia* cysts. Raw water turbidities ranged from <0.1 to 60 NTU at one location and <0.1 to 101 NTU at the other. The authors suggested that the low removal was due to poor coagulation. Even though the coagulation process was not optimized and cyst removal was poor, the finished water turbidity was less than 0.5 NTU.

Three Montreal area water treatment facilities that used conventional filtration with ozone disinfection were sampled for a number of pathogens and indicator microorganisms at various locations (raw, settled, filtered water) by Payment and Franco (1993). *Giardia* cysts were detected in 80%-100% of raw water samples with geometric mean levels of cysts at each of the three locations 7.23, 336, and 1376 per 100 L, respectively. *Giardia* cysts were detected in only

one sample of filtered water -- at the treatment plant with the highest level of cysts. Based on a geometric mean of 0.1 *Giardia* cysts per 100 L in filtered water at this plant, a removal of 5.2 $\log_{10}$ cysts was calculated. At the other two plants >5 $\log_{10}$ removals of *Giardia* cysts were calculated based on analytical detection limits. Sedimentation at the plants was shown to remove 2.7-2.9 $\log_{10}$ of *Giardia* cysts.

States et al. (1995, 1997) reported that the Pittsburgh Water Treatment Plant effectively removed *Giardia* cysts from the Allegheny River source. Based on the relatively low arithmetic mean of *Giardia* in the Allegheny river source, removal of cysts was calculated to be 1.7 $\log_{10}$; the removal based on the geometric mean was 1.5 $\log_{10}$. Although no cysts were detected in filtered water, filter backwash water samples showed positive occurrences of *Giardia* cysts on 13% of the sampling occasions and in 8% of the water samples, with arithmetic and geometric means of 16.8 and 58.6 cysts/100 L of filter backwash water, respectively. This emphasizes the importance of backwash water as a source of contamination whether it is disposed or recycled to the influent of the treatment plant. Proper management including treatment, equalization of flow, and monitoring is required when backwash water is recycled (Cornwell and Lee, 1994).

In summary, studies indicate that conventional and direct filtration, when operated under appropriate coagulation conditions, can remove 3 to 4 $\log_{10}$ of *Giardia* cysts. The highest removal rates occurred in pilot plants and water systems where coagulation was optimized and low filtered water turbidities (0.1- 0.3 NTU) were achieved. In plants where coagulation was not optimized, cyst removal was poor even when low turbidities were achieved in filtered water. High levels of cysts are found in filtered backwash water, and this source of contamination should be considered before backwash water is discharged or recycled.

b. Slow Sand and Diatomaceous Earth filtration

Jakubowski (1990) reported data from various studies which indicated that both DE and slow-sand filtration are effective in removing *Giardia* cysts from water. A well designed and operated plant using slow-sand or DE filtration is capable of removing at least 3 $\log_{10}$ of *Giardia*. Removal is less efficient for slow-sand filters at near freezing temperatures (Fogel et al. 1993; Schuler et al., 1991). Cleasby et al. (1984) found that when source water quality is high, slow sand filtration outperforms direct filtration with alum or cationic polymer as a coagulant. Direct filtration also required substantially more operational skill and attention. Slow sand filtration is particularly appropriate for small water treatment systems where there may be limited operating personnel present on-site for continuous monitoring of filter efficiency. Riesenberg et al. (1995) found a slow sand filter in Camptonville, California, satisfactorily maintained filtered water turbidity levels of <1.0 NTU despite stream turbidities of >30 NTU.

Bellamy et al. (1985) reported the results of pilot-scale studies which showed slow sand filtration was capable of removing virtually 100% of *Giardia* cysts as the sand bed matures. At hydraulic loading rates of 0.04 to 0.4 m/h, human-source *Giardia* cyst removals were uniformly high and averaged > 3 to 4 $\log_{10}$. Fogel et al. (1993) reported on the efficiency of removal for a slow sand filtration system in British Columbia where no detectable cysts were found in 34 of 35 filter effluent samples. One sample contained a single cyst (11 cysts per 100 L). Based on this limited sampling, the slow sand filter was reported to be able to remove an average of only 93% of the *Giardia* cysts found in the raw water. Schuler et al. (1991) reported data from pilot-scale studies of slow sand and DE filtration of water with turbidities ranging from 0.1 to 5.8 NTU. Results indicated that both types of filters were able to remove greater than 3 $\log_{10}$ of *G. muris* cysts; however, only 2-3 $\log_{10}$ removals could be achieved in the slow sand filter during the winter months and removal efficiency of the DE filter was decreased during a malfunction that caused the filter cake to crack.

Logsdon et al. (1981) evaluated the removal of 9- m-diameter radioactive microspheres and *G. muris* cysts by DE filters. DE filtration consistently removed >2 $\log_{10}$ of microspheres

and cysts and frequently achieved >3 $\log_{10}$ removal. Effective filtration was dependent on DE precoat thickness up to 1.0 kg/m^2 precoat of diatomite. Effluent turbidity was not found to be an effective indicator of DE filtration efficiency, and thus, reliance of effective removal depends solely on proper operation. Subsequent studies with human-source *Giardia* cysts confirmed that DE filtration could remove >2 $\log_{10}$ of cysts (Jakubowski, 1990). Pilot plant studies by Lange et al. (1986) showed that virtually 100% (qualified by detection limits) of *G. lamblia* cysts were removed by DE filtration. Water temperature did not affect DE filter performance but finer size DE and lower filtration rates resulted in higher removal of bacteria and turbidity. DE filtration is effective for *Giardia* cyst removal; however, the raw water must be of low turbidity and good microbial quality, and the DE filter must be operated properly (Logsdon, 1988).

c. Membrane and Other Filters

At least 2 $\log_{10}$ removal of *Giardia* cysts should be possible with various types of point-of-use/point-of-entry (POU/POE) systems employing such devices as cartridges containing materials such as yarn-wound fibers, ceramics, paper, or other types of filtration media of an appropriate effective size (Jakubowski, 1990). However, to adequately protect against waterborne disease, systems should be selected based on their capability to remove 3 $\log_{10}$ *Giardia* cysts or cyst-sized particles, and it should be remembered that smaller-sized protozoa may not be removed by systems that can effectively remove *Giardia* cysts. Jacangelo et al. (1995) studied the removal of *G. muris* by two hollow fiber microfiltration membranes, one spiral wound fiber microfiltration membrane, two hollow fiber ultrafiltration membranes, and one tubular ceramic ultrafiltration membrane. Nominal pore sizes of the microfiltration membranes were 0.1 to 0.2 µm, and nominal molecular weight cutoffs of the ultrafiltration membranes were 100,000 to 500,000 daltons. The membranes were found to achieve from 4.6 to >5.2 $\log_{10}$ removals of cysts under bench-scale worst case operating conditions, and >6.4 $\log_{10}$ removals of cysts under pilot plant normal operating condition. All of the hollow-fiber membranes removed *G. muris* cysts to less than detectable levels; no cysts were detected as long

as the membrane remained intact. Physical straining of cysts appeared to be the primary mechanism of filtration. Earlier studies by Jacangelo et al. (1991) also found good removal of *G. muris* cysts by low-pressure hollow fiber ultrafiltration membranes; 4.1 to 5.0 $\log_{10}$ removals were obtained from four different source waters -- two from northern California with mean turbidities of 0.5 and 9 NTU and two from Boise, Idaho with mean turbidities of 0.5 and 4.9 NTU. A pilot study to determine the feasibility of reclaiming municipal wastewater found an 8-10 log removal of *Giardia* by ultrafiltration/nanofiltration membranes (Madireddi et al., 1997).

2. Disinfection

Jakubowski (1990), Hoff (1986) and Jarroll (1988) reviewed the effectiveness of disinfectants to inactivate *Giardia* cysts. These reviews considered only studies where in vitro excystation or animal infectivity was used to assess cyst viability or infectivity because these are more sensitive indicators than other methods, such as the ability of cysts to exclude vital stains (Bingham et al., 1979). Hoff et al. (1985) compared animal infectivity and excystation as endpoints for determining the efficacy of disinfection of *Giardia muris* cysts; viability was assessed before and after exposure to free residual chlorine. Substantial inactivation of cysts was observed by both mouse infectivity and excystation after exposure to an initial free chlorine residual of 1 mg/L at pH 7.0, at 5°C, and Hoff et al. (1985) concluded that in vitro excystation was an adequate indication of *G. muris* cyst infectivity. Studies by Rice et al. (1982) found that *G. muris* cysts were more resistant to inactivation by chlorine than human-source cysts and thus, should provide a conservative indication of disinfection effectiveness. Hoff et al. (1985) also found that *Giardia muris* cysts were more resistant than human-source cysts to free chlorine. Human-source *Giardia* cysts for chlorination studies can be obtained from either asymptomatic or symptomatic persons, since Rice et al. (1982) found that they have similar resistance to chlorine. Information from a number of early disinfection studies indicated that *G. muris* were

among the most resistant waterborne microorganisms to chlorine and other disinfectants (Jakubowski, 1990).

Results of studies by Jarroll et al. (1981) are a reminder that chlorine does not always result in 100% inactivation of *Giardia* cysts. Jarroll et al. (1981) studied chlorine inactivation of human-source *Giardia* cysts at water temperatures of 5°C, 15°C, and 25°C, water pH of 6, 7, and 8, chlorine contact times of 10, 30, and 60 minutes, and chlorine concentrations from 1 to 8 mg/L. The inactivation of cysts by chlorine was found to be less effective at higher pH values, and lower water temperatures. Less than 30% of cysts were inactivated at water temperatures of 5°C and exposures to 2 mg/L chlorine for 30 minutes contact time at pH 8. Further, at water temperatures of 5°C, exposures to 1 mg/L chlorine for 10 minutes contact time at pH 8 less than 45% of cysts were inactivated. At the time of this study, many unfiltered surface water systems in the United States used similar contact times and chlorine concentrations for these water temperatures and pH values suggesting that chlorination was inadequate in many water systems, especially those that reported waterborne outbreaks. At 25°C, exposure to 1.5 mg/L chlorine for 10 minutes killed all cysts at pH 6, 7, and 8. At 15°C, 100% mortality required exposure to 2.5 mg chlorine/L for 10 minutes at pH 6; however, at pH 7 and 8, small numbers of cysts (less than 0.8%) remained viable after 30 minutes while no cysts were viable after 60 minutes. At 5°C, exposures to 1 mg/L chlorine for 60 minutes did not kill 100% of the cysts at any pH tested, while 2 mg/L resulted in 100% mortality of the cysts after 60 minutes at pH 6 and 7 but not at pH 8. A chlorine concentration of 4 mg/L also caused 100% mortality at all three pH values after 60 minutes but not after 30 minutes. Chlorine concentration of 8 mg/L killed 100% of the *Giardia* cysts at pH 6 and 7 after contact for 10 minutes but required 30 minutes exposure at pH 8.

Hoff (1986) calculated *Ct* values for 99% inactivation of *Giardia* cysts by chlorine using published and unpublished data (Table VII-2). *Ct* is the product of the concentration (C) of a disinfectant (mg/L) and its contact time in minutes (t). A low *Ct* value indicates more effective disinfection. For example at 15°C, a *Ct* value of 20 (pH 6) is almost twice as effective as a *Ct*

value of 37 (pH 8). In general, the effectiveness of chlorination was found to increase considerably at higher water temperatures and at lower pH values. The most pronounced pH effect on chlorination of human-source *Giardia* cysts was seen at lower water temperatures. Jakubowski (1990) summarized extensive investigations reported in 1987 by Hibler et al. conducted of the inactivation of human-source *Giardia* cysts by chlorine at various water temperatures, pH, contact times, and concentrations (Table VII-3). Infectivity of Mongolian gerbils was the end-point studied. Experimental water temperatures were selected based on the temperatures of water sources in areas where most outbreaks were being reported. Similar to the findings of Hoff (1986) and others, chlorination was found to be less effective at lower water temperatures and pH. Also noted were erratic results in experiments with chlorine concentrations above 2.5 mg/L and suggested that *Ct* values calculated with high chlorine concentrations may not be reliable (Jakubowski, 1990).

Table VII-2. *Ct* Values for 99% Inactivation of *Giardia* cysts by Free Chlorine

Water Temp.	pH	Chlorine (mg/L)	Time (min)	Mean *Ct*	Source of Cysts
3°C	6.5 7.5	0.24-1.1 0.24-1.0	37-297 150-770	68 140	*G. muris* *G. muris*
5°C	7	0.41-2.73	236-467	360	*G. muris*
5°C	6 7 8	1.0-8.0 2.0-8.0 2.0-8.0	6-84 7-152 57-164	65-75 97-118 110-142	human human human
15°C	6 7 8	2.5-3.0 2.5-3.0 2.5-3.0	7 6-18 7-21	20 32 37	human human human
25°C	5 7 9	4.4-13 2.9-7.1 11.6-72.6	4-16 4-16 3-16	66 29 206	*G. muris* *G. muris* *G. muris*
25°C	6 7 8	1.5 1.5 1.5	<6 <7 <8	<9 <10 <12	human human human

*Adapted from Hoff (1986) and Jakubowski (1990).

Jarroll (1988) reviewed the inactivation data available for chlorine, chloramines, chlorine dioxide, ozone, ultraviolet (UV) irradiation, and iodine. *Ct* values were presented for the chemical disinfectants considering the effects of water temperature, pH, disinfectant concentration, contact time for each disinfectant. Ozone, chlorine dioxide, free chlorine, iodine, and chloramines, listed in descending order of effectiveness, were judged to be effective for *Giardia* cysts. Jarroll (1988) cautioned that, while *G. muris* had in every disinfectant tested up to that time been more resistant than *G. lamblia* cysts, the kinetics of the pH effect on chlorine disinfection had recently been found by Leahy et al. (1987) to be different between *G. muris* and *G. lamblia* cysts and contrary to earlier work.

Table VII-3. *Ct* Values for 99.9 to 99.99% Free Chlorine Inactivation of Human-Source *Giardia* cysts (Mongolian Gerbil Infectivity Assay)

Water Temp.	pH	Mean *Ct* Values
0.5°C	6-8	185-342
2.5°C	6-8	142-268
5.0°C	6-8	146-280

*Adapted from Hibler et al. (1987) and Jakubowski (1990).

Rubin et al. (1989) evaluated the inactivation of human-source *Giardia* cysts by free chlorine using Mongolian gerbils; *Ct* values were found to be higher than previously reported with lower *Ct* values at higher pH levels. At 15°C the *Ct* ranged from 5-62 at pH 9 compared to *Ct* values of 139-182 at pH 5. Jakubowski (1990) reported that Rubin also found that *Ct* values for *G. muris* cyst inactivation by preformed monochloramine were substantially higher than those for chlorine at pH 7 and 5°C. Meyer et al. (1988) found lower *Ct* values than Rubin for inactivation of *G. muris* cysts by chloramines as they are being formed, but *Ct* values were still

found to be much greater than those for chlorine. Jarroll (1988) found that *G. muris* cysts were more resistant to chloramines at lower pH values and that preformed chloramines were less effective than chloramines that are not preformed. Hofmann and Andrews (1995) reported data from disinfection experiments that indicated inactivation of *Giardia* cysts was more efficient at pH 6.5 than 8.5, at 25°C than at 5°C, and that chlorine is more effective than chloramines. These pH values were selected to be representative of typical water sources in Ontario, Canada, with water temperatures representing winter and summer conditions.

Finch et al. (1995) summarized the Leahy's (Master of Science thesis, Ohio State university, 1985) evaluation of chlorine dioxide inactivation of *G. muris* cysts using in vitro excystation as an indicator of viability. Chlorine dioxide was an order of magnitude more effective than free chlorine at 25°C and two orders of magnitude more effective at pH 9. In contrast to findings with chlorine, chlorine dioxide effectiveness increased at higher pH values (Jakubowski, 1990). At 25°C, the *Ct* value for chlorine dioxide ranged from 4.9-6.9 at pH 5 compared to a *Ct* of 1.7-3.0 at pH 9 (Leahy, 1985). In a pilot-scale study of *G. muris* inactivation, a *Ct* value of 12 was reported for 99.9% inactivation at pH 8 and 8°C; viability was determined by animal infectivity and in vitro excystation with similar results for each (Finch et al., 1995).

Giardia cysts are readily inactivated by ozone (Wickramanayake et al. 1984a, b, 1985; Wolfe et al., 1989; Labatiuk et al., 1991; Finch et al., 1993; Owens et al., 1994). Wickramanayake et al. (1984a, b, 1985) found ozone to be more effective than chlorine for inactivation of either human-source *Giardia* or *G. muris* cysts and less affected by water temperatures. *G. muris* was slightly more sensitive to ozone at pH 5 than at pH 7, but was nearly one and one-half times more resistant at pH 9 (Wickramanayake et al., 1984b). Finch et al. (1993) found that the resistance of *G. lamblia* to ozone was not significantly different from that of *G. muris* at 22°C and contact times of 2 and 5 minutes. Viability was assessed by the C3H/HeN mouse and Mongolian gerbil models for G. lamblia and *G. muris*, respectively. The

Ct value for 99.9% inactivation of *G lamblia* by ozone was found to be 2.4 times greater than the recommended *Ct* value in the SWTR Guidance Manual (U.S. EPA, 1989).

After comparing animal infectivity, excystation, and fluorogenic dye as measures of cyst inactivation by ozone, Labatiuk et al. (1991) concluded there were no significant differences among the three methods for inactivations up to 99.9%; however, only the C3H/HeN mouse model had the sensitivity to detect inactivations greater than 99.9%. Labatiuk et al. (1992) also found that water temperature, pH, and applied/residual ozone dose were important factors affecting inactivation of *G. muris* cysts. Contact times of up to 2 minutes had a significant effect in demand-free buffered water, but contact times up to 5 minutes were required for inactivation in natural waters suggesting caution in applying results of laboratory disinfection studies to natural waters. It was also found to be more difficult to achieve 99 or 99.9% inactivation of cysts in natural waters at 22°C than 5°C. Haas and Heller (1989) studied the experimental data of Hibler (1988) for the inactivation of *Giardia* by free chlorine and recommended caution against extrapolation of inactivation results outside the range of experimental conditions. Haas et al. (1996) also cautioned that inactivation data obtained in laboratory studies using buffered demand-free water do not appear to be adequate for predicting inactivation in actual waters to be disinfected. In earlier studies, Haas et al. (1994) determined that *Giardia* inactivation can be achieved with free chlorine, monochloramine, and ozone in buffered demand-free water, as well as in waters from two rivers, at dosages similar to those that might be employed in water treatment facilities, providing the concentrations are high enough.

Hydrogen peroxide is sometimes added to ozone for oxidation of organic compounds. Bench scale studies of the inactivation of *G. muris* with ozone and ozone-hydrogen peroxide showed significantly greater inactivations in the presence of an ozone residual, leading Labatiuk et al. (1994) to conclude that the design of ozone disinfection processes should maintain an ozone residual for disinfection before the addition of hydrogen peroxide.

An on-site disinfection process for small communities uses electrolysis of a sodium chloride solution to produce a mixture of oxygen and chlorine species (mixed oxidant gases or MOGOD). Witt and Reiff (1997) presented data showing the effectiveness of MOGOD; *Ct* values were comparable to those for ozone and chlorine dioxide (Table VII-5).

Rice and Hoff (1981) evaluated UV irradiation for inactivation of human-source *Giardia* cysts. They found a reduction of less than 90% of *Giardia* cysts at the maximum dose tested, 63,000 W-s/cm^2 of UV irradiation at a wavelength of 254 nm. Both human-source *Giardia* and *G. muris* cysts are significantly more resistant to ultraviolet irradiation than *E. coli* and *Yersinia* sp. (Rice and Hoff, 1981; Jakubowski, 1990; Karanis et al., 1992). Karanis et al. (1992) noted that UV disinfection is not reliable because commercial use a dose range of 250-350 J/m^2; a 2 $\log_{10}$ reduction of *G. lamblia* cysts required 1800 J/m^2.A new generation of UV irradiation devices with improved disinfection capabilities are currently being evaluated for their ability to inactivate *Cryptosporidium* oocysts; however, no studies have been reported for *Giardia*.

Clark et al. (1989) and Clark (1990) described the development of a model based on first-order kinetics to relate *Ct* values from inactivation data to free chlorine concentration, pH, and temperature for use by water utilities in meeting provisions of the EPA's SWTR that requires 99.9% reduction of *Giardia* cysts from surface water sources. The *Ct* values of the SWTR Guidance Manual (U.S. EPA, 1989) were based on a number of simplifying assumptions, such as the Chick-Watson relationship for microbial inactivation and extrapolated values from data that had been obtained from laboratory studies in buffered demand free water. Haas et al. (1996) cautioned that the type of source water may be significantly affect predictions for microbial inactivation. Factors other than disinfectant demand appear to influence inactivation of organisms in natural waters, inactivation data obtained in laboratory studies using buffered demand-free water do not appear to be adequate predictors of inactivation in actual waters to be disinfected, and the use of pH in adjusting *Ct* values is insufficient to characterize the effect of water quality on disinfection performance (Haas et al., 1996). Haas and Joffe (1994) proposed an

approximation to the exact solution of Hom's model for microbial disinfection kinetics to describe inactivation in batch systems subject to disinfection decay/demand. The approximate and exact solutions were found comparable for *G. muris* cysts exposed to preformed monochloramine concentrations of 1 and 2 mg/L at pH 6.9 and 18ºC in water obtained from the Bull Run Reservoir.

Table VII-5. Effectiveness of Water Disinfectants for 99% Inactivation of *Giardia* Cysts

Disinfectant	Temp	pH	*Ct*	Cysts	Reference
Ozone	25°C	7	0.3	*G. muris*	Wickramanayake et al., 1984b
Ozone	5°C	7	1.9	*G. muris*	Wickramanayake et al., 1984b
Ozone	25°C	7	0.2	Human	Wickramanayake et al., 1984a
Ozone	5°C	7	0.5	Human	Wickramanayake et al., 1984a
MOGOD	20°C	6-7.5	3	Human	Witt & Reiff, 1996
MOGOD	3-5°C	6-7.5	6-10	Human	Witt & Reiff, 1996
Chlorine Dioxide	25°C	7	5	*G. muris*	Jarroll, 1988
Chlorine Dioxide	5°C	7	11	*G. muris*	Jarroll, 1988
Free Chlorine	25°C	7	26-45	*G. muris*	Leahy et al., 1987; Jakubowski, 1990
Free Chlorine	5°C	7	360-1012	*G. muris*	Leahy et al., 1987; Jakubowski, 1990
Free Chlorine	25°C	7	<15	Human	Jarroll et al., 1981; Jakubowski, 1990
Free Chlorine	15°C	7	120-236	Human	Rubin et al., 1989
Free Chlorine	5°C	7	90-170	Human	Jarroll et al., 1981; Rice et al., 1982; Jakubowski, 1990
Chloramine	18°C	7	144-246	*G. muris*	Jarroll, 1988
Chloramine	3°C	7	425-556	*G. muris*	Jarroll, 1988
Preformed Chloramine	15°C	7	825-902	*G. muris*	Jarroll, 1988
Preformed Chloramine	5°C	8-9	1400	*G. muris*	Witt & Reiff, 1996

Because of the high risk of waterborne giardiasis among campers, hikers, backpackers, and travelers to developing countries, personal water purification methods employing iodine or chlorine disinfection were reviewed by Jarroll (1988). At water temperatures of 20°C, all of the six disinfection methods tested by Jarroll (1988) were completely effective against *G. lamblia* cysts when prescribed procedures were employed for cloudy and clear water. However, at 3°C several methods failed to completely inactivate cysts suggesting that procedures (residual concentrations and contact times) be revised for low water temperatures. Ongerth et al. (1989b) found that for seven disinfection methods tested, iodine-based methods were more effective than chlorine-based methods; however, no chemical treatment achieved 99.9% cyst inactivation after 30 minutes. Heating water to at least 70°C for 10 minutes was found to be an acceptable alternative (Ongerth et al., 1989b).

IV. Summary

1. Analysis

The absence of a practical cultural method for *Giardia* in environmental samples, and the probability that one could not be developed, led to the development of microscopic examination assay methods. Since *Giardia* does not reproduce in the environment once it leaves the host, large-volume sample collection methods were developed using filtration through microporous cartridge media. Collecting large volume samples of raw source water resulted in many eluates containing a significant amount of particulates that had been retained on the filters. Initially, flotation clarification techniques used zinc sulfate solutions; subsequently, other compounds including sucrose, Percoll, and Percoll-sucrose were evaluated and incorporated into the method. The development of fluorescent antibodies for *Giardia* revolutionized the detection step which had previously been dependent upon examining concentrates with none-selective iodine staining. A combination method was also developed whereby a single sample could be simultaneously assayed for *Giardia* and *Cryptosporidium*.

The original *Giardia* method was developed to assist in waterborne outbreak investigations. It subsequently was adapted to different applications by those with a need to determine drinking water treatment effectiveness, or occurrence and distribution of cysts in the environment, or to study fate and transport of cysts. In the absence of regulatory requirements to monitor for *Giardia*, there was no official standardized method. However, voluntary efforts through groups such as *Standard Methods for the Examination of Water and Wastewater* and ASTM resulted in consensus reference or proposed methods that could be used as a baseline and modified as needed for particular applications.

The availability of consensus methods resulted in evaluation studies of all steps involved in the methodology including sampling, elution, flotation clarification, and microscopic assay. The sample collection and elution steps were found to account for significant losses of cysts. In addition, aspects of flotation clarification, especially the specific gravity of the gradient solution and the relative centrifugal force used to spin samples, were found to significantly affect recovery. While retention of cysts and oocysts on the sampling filter was improved by higher turbidities in the water being sampled, the greater quantity of material obtained in the sample pellets presented difficulties in the flotation purification and microscopic assay steps. The nature of the turbidity (e.g., organic or inorganic, particle size, etc.) was more important than the total amount in causing detection and identification problems. For example, algae could make clarification and detection more difficult in certain types of water and at certain times of the year.

The fluorescent antibody assay, while improving detection of cysts, necessitated developing a new definition for identifying cysts. Presumptive cysts were defined by size, shape and apple green fluorescence under specified conditions of reagent type and use and microscope configuration; confirmed cysts were those meeting the presumptive criteria plus having defined internal characteristics. This terminology created confusion for interpreting results, especially by those not familiar with the methodology, who often ignored the results if no confirmed cysts were found. The presumptive designation included all objects that might be *Giardia* cysts. The

confirmed designation was applied to those presumptive cysts that could definitely be identified as *Giardia*. The remaining objects might or might not be *Giardia* because interferences, e.g., cross-reactions, were known to occur. Some cysts in a known, purified preparation of *Giardia* will not meet the criteria for confirmation. The presumptive/confirmed terminology was replaced with total counts/counts with internal structures in the *Standard Methods* and ICR methods. Another limitation of fluorescent antibody identification is that it may only be specific to the family level. While antibodies with various specificities have been developed, the application and interpretation of results with them is complicated by uncertainty in defining species within the genus, and in identifying those species that might have public health significance.

Nucleic acid-based detection and identification techniques have been developed. While they have the potential to specifically detect those species that may be important in human infection, and they have demonstrated sensitivity down to one cyst in purified preparations, they have yet to realize their full potential. Problems have been encountered with reproducibility of the assays and with inhibition of the PCR reaction in environmental samples.

The advent of the ICR, and the necessity for developing defined data quality objectives for that monitoring effort, resulted in performance evaluation data that underscored the low precision of the method in unapproved laboratories. With the promulgation of the ICR, for the first time a process was implemented in the United States for approving and conducting continual performance evaluation of analysts and laboratories that wished to do environmental protozoa analyses. Until that time, adherence to specific methodological protocols, or performance of recommended quality assurance/quality control procedures, was strictly voluntary. Maintaining a similar process after completion of the ICR may help to ensure the reliability of data obtained through continued monitoring efforts.

Increased awareness of method limitations has also spurred development of alternative methods and procedures. In the area of sample collection, sampling 10 L volumes instead of 100 L or more for raw waters is being investigated. Processing the entire concentrate for a 10 L sample may be preferable to processing an undefined portion of a 100 L sample in terms of the detection limit and it could help the laboratory and drinking water treatment utilities interpret their results. Collecting smaller sample volumes means fewer particulates to cause interferences in the detection assay, and it makes it easier to apply alternate separation technology (instead of flotation separation where cyst recovery is low or erratic), such as immunomagnetic techniques. Also, the use of membrane filters with defined porosity (instead of yarn-wound filters with nominal porosities) for sample collection can result in better recoveries. For the assay portion of the methodology, much of the tedium and fatigue associated with examining concentrates may be relieved by using techniques such as flow cytometry and cell sorting.

Dependence upon non-cultural methods for the detection and identification of *Giardia* in environmental samples has rendered determining the public health significance of positive findings problematical. Determining the viability or infectivity potential of small numbers of cysts detected with non-cultural methods has been difficult or impossible to do. A detected cyst may be either viable (alive) or non-viable (dead). Viable cysts are not necessarily infectious. If the organism is alive but has been injured, it may not be infectious. While viability determinations might not be necessary for some applications, such as waterborne outbreak investigations or determining the effectiveness of a treatment process to physically remove cysts, they are very important in developing risk assessments upon which to base treatment requirements or drinking water regulations.

Procedures used to determine viability have included dye staining, morphological criteria, in vitro excystation, animal infectivity, and nucleic acid-based assays. Traditional dye staining methods (e.g., with eosin) were found not to correlate with in vitro excystation or animal infectivity. Subsequent research produced dyes that enter the viable cyst (e.g., FDA) and those

that are excluded from the viable cyst while they can enter non-viable cysts (e.g., PI). Work that has been done with PI to date indicates that cysts stained with this compound are not viable. However, cysts that do not take up the stain may be either viable or non-viable, and whether or not inactivated cysts stain may depend in part on how they were inactivated.

At least with *G. muris*, morphological criteria have been shown to correlate with PI staining and animal infectivity. Clearly defined internal characteristics and the absence of a peritrophic space are indicative of non-viable cysts. In vitro excystation also works well with *G. muris* but it is erratic with *G. lamblia* cysts. Another problem is that while excystation may be a good measure of viability for determining disinfectant effectiveness where large numbers of cysts are used in an experimental design, the procedures are not practical for application to the small numbers of cysts likely to be detected in water samples.

Dye staining, morphological criteria, and in vitro excystation may be adequate indicators of viability for some applications but could be conservative in estimating the potential for infection. Animal infectivity has commonly been used in experiments to determine disinfectant efficacy. However, it has seldom been used to evaluate the health significance of environmental isolates. An exception would be the Canadian studies described by Wallis et al. (1996). They concluded that gerbil animal infectivity can be inaccurate because some strains do not infect gerbils. There is also the possibility that infectious cysts below the infectious dose might be present and not detected. Other problems with this method are the cost and the necessity for maintaining approved laboratory animal facilities.

Nucleic acid-based viability assays have focused on the detection of mRNA by RT-PCR techniques using either the giardin gene or an HSP gene. Amplification of the HSP gene has not proven reliable and there is some question about the survival and longevity of mRNA when the organism is inactivated by different techniques. Besides practical problems relating to the

sensitivity and application of PCR techniques to environmental samples, the question of how viability determined by these techniques relates to infectivity remains to be resolved.

For diagnosis of giardiasis in either humans or animals, stools continue to remain the specimen of choice. In humans, the majority of infections can be detected by stool examination, but in some instances, examination of duodenal or intestinal fluids (by aspiration, biopsy or string test) or the use of radiological procedures may be necessary. Fresh stools can be used to prepare wet mounts that are examined by conventional light microscopy for the presence of cysts or trophozoites.

Fresh, frozen, or preserved stools can be examined using traditional dye staining techniques or with increasingly popular immunofluorescence assays. A variety of commercially-available fluorescent antibody kits that target cysts or antigens are available. Evaluation of these kits indicates that they have a high degree of sensitivity and specificity. They may require less time to perform and produce results with a single stool sample equivalent to fresh stool and dye staining techniques that require multiple stool examinations. The use of flow cytometry with immunofluorescence reagents may allow a greater number of human or animal specimens to be examined in a given time period with less operator fatigue.

For surveys of giardiasis in animal populations, examination of intestinal scrapings from live-trapped animals may prove more fruitful than examination of feces from kill-trapped animals. With either human or animal specimens that have been frozen and thawed before examination, immunofluorescence assays are more likely to detect cysts than is examination by conventional microscopy. This may allow samples to be archived and subsequently re-examined for a variety of purposes, including quality control. One author concluded that phase microscopy had an advantage over immunofluorescence assays in that phase microscopy allowed some differentiation to the species level of cysts found in wild rodent populations.

Serodiagnosis is still not a useful technique in the clinical environment due to the inability to distinguish between present and prior infections. However, serologic testing may have value in conducting epidemiological studies. Secretory antibody has been detected in a small study of saliva specimens from patients infected with *Giardia*. The potential for developing tests that could be useful for either diagnostic or epidemiologic purposes based on this finding remains to be determined. Also, the development and application of gene probe techniques (e.g., PCR) for clinical diagnostic purposes has thus far proved challenging due to inhibitory substances in feces and resulting problems with sensitivity and specificity.

2. Water Treatment

Information obtained during the past 20 years from laboratory, pilot plant, and full scale treatment plant studies show that *Giardia* cysts can be effectively removed and inactivated by a combination of filtration and disinfection. Because of *Giardia*'s low infectious dose, the wide-spread occurrence of the infection in humans and a variety of animals, and the relative resistance of *Giardia* cysts to environmental conditions and water disinfectants, it is important to consider multiple barriers for the protection and treatment of both surface and ground water sources: a combination of watershed protection for surface waters and well-head/aquifer protection for ground water sources, water filtration, disinfection, and protection of the integrity of the distribution system. Use of all of these barriers affords the most effective means for assuring the microbial safety of public water supplies (Geldreich and Craun, 1996).

It is impossible and morally unacceptable to eliminate animal sources of contamination from a watershed. However, sources of contamination from farming and domestic animals can be controlled and sources of contamination from wild animals and their impact on surface water sources can be reduced. Control of human sewage discharges and appropriate wastewater treatment will also help reduce contamination of source waters. The majority of waterborne giardiasis outbreaks have occurred in unfiltered surface water systems, but outbreaks have also

occurred in groundwater systems impacted by surface water or sewage discharges. Wells and springs should be protected from the influence of surface water and sewage discharges from septic tanks and municipal wastewaters. While these controls can reduce the potential for contamination of source waters, they cannot eliminate it. To effectively protect against the waterborne transmission of *Giardia*, water treatment barriers are required. For surface water sources and groundwater sources under the influence of surface water, disinfection and filtration are also recommended. Filtration exceptions may be granted where water sources meet criteria of the EPA's SWTR; however, if water sources are subject to contamination with *Cryptosporidium*, it should be remembered that disinfection levels used to inactivate *Giardia* cysts may not be sufficient to inactivate *Cryptosporidium* oocysts (Craun, 1997) and filtration may be necessary.

Filtration technologies commonly used by water utilities can be designed and operated to remove 99% or more of *Giardia* cysts. Studies indicate that conventional and direct filtration, when operated under appropriate coagulation conditions, can remove 3 to 4 logs $\log_{10}$ of *Giardia* cysts. The highest removal rates occurred in pilot plants and water systems that optimized coagulation and achieved very low finished water turbidities (0.1- 0.3 NTU).

Disinfection is an important part of the multiple barrier concept of water treatment. Chemical disinfectants can also reduce cyst densities, but the effectiveness of disinfection can be affected by water temperature and pH, applied and residual disinfectant concentration and contact time, particles that can shield cysts from contact with the chemical, and organic matter that can cause disinfectant demand. Filtration can make disinfection more effective by reducing the disinfectant demand and removing particles that may interfere with disinfection effectiveness.

Disinfection can also achieve 99% or greater inactivation of *Giardia* cysts: however, the EPA regulates disinfectants and disinfection by-products and this may limit the concentration and contact time of any chemical disinfectant that can be applied. When lower concentrations of a

disinfectant are required to meet disinfection and disinfection by-product limits, both filtration and disinfection may be necessary so that sufficient levels of *Giardia* cysts are removed to prevent the waterborne transmission of giardiasis.

The majority of waterborne giardiasis outbreaks have occurred in unfiltered surface water systems or unfiltered ground water systems impacted by surface water or sewage discharges. This emphasizes the need for water filtration. Outbreaks have also occurred in filtered water systems, and this shows the need for good design, optimization of the filtration process(es), and frequent monitoring of treatment effectiveness. Both conventional and direct filtration facilities should be designed with proper chemical pretreatment to provide adequate coagulation. In some source waters, sedimentation may be needed to effectively remove cysts. In water filtration plants where coagulation was not optimized, cyst removal was poor even though relatively low turbidities were achieved in filtered water. High levels of cysts are found in filtered backwash water, and this potential source of contamination should be considered before this water is discharged to the environment or recycled back to the beginning of the water treatment plant.

Chemical disinfection employed by the water industry can inactivate *Giardia* cysts; however, *Giardia* can be resistant to low doses of chlorine and chloramines, and there are differences between the inactivation efficiencies of the various disinfectants. The reported effectiveness of inactivation by the typically utilized disinfectants, in decreasing order of efficiency, is follows: ozone, MOGOD, chlorine dioxide, iodine, free chlorine, and chloramines. UV irradiation does not appear to be useful under current operating conditions. Water quality including temperature and pH are important factors to consider when selecting a disinfectant and its concentration and contact time. The turbidity and disinfectant demand of the water to be disinfected are also important concerns. In waters of high turbidity the effectiveness of a disinfectant may be greatly reduced. *Ct* values are available to compare disinfectants, and values are recommended for various conditions of temperature and pH. Applied and residual concentrations are important to consider as is how disinfectants are added. For example, ozone-

peroxide is less effective than ozone and preformed chloramines are less effective than chloramines that are not preformed. Information is available to provide guidance in selecting *Ct* values, but it must be remembered that source water quality is also important. Since published *Ct* values are based on results of laboratory studies in demand-free water, caution is recommended in extrapolating of these data to natural waters and beyond the experimental conditions. It should also be remembered that if source waters are heavily contaminated with *Giardia* cysts, disinfection alone may not be sufficient to protect against waterborne infection. Disinfection may be adequate to inactivate 99.9% of the cysts, but the remaining cysts may still be at infectious dose levels.

VIII. RECOMMENDATIONS FOR ADDITIONAL RESEARCH

F. Chapter II. General Information and Properties

Additional information is required to answer questions about *Giardia* species and zoonotic routes of transmission. The characterization of *Giardia* by molecular approaches, such as zymodeme or karyotype identification, can be useful in this regard. To conclusively determine whether human giardiasis can be acquired by zoonotic routes and whether the ultimate source was human or a lower animal will require carefully controlled animal feeding studies and more comprehensive epidemiological investigations. Epidemiological investigations of outbreaks and endemic risks, especially waterborne, should include the systematic collection of *Giardia* cysts from infected humans, from animals suspected of transmission, and from environmental samples and their characterization by molecular approaches.

G. Chapter III. Occurrence

A significant database has been developed on the occurrence and distribution of *Giardia* in a variety of waters including municipal wastewater, surface water, and groundwater. Another large database of occurrence information is being developed through water monitoring required by the ICR. However, the sources of contamination on specific watersheds and the factors affecting fate and transport of cysts are not as well characterized. For example, giardiasis is more common in immature animals, and studies should be conducted to determine water quality changes associated with the reduction and relocation of suspected animal sources, such as beaver. As beaver begin to repopulate the watershed, their average age distribution will be much younger with perhaps an accompanying larger prevalence of infection and greater contribution to source water contamination.

There is no published information on the occurrence of *Giardia* in soils and sediments, probably due to the difficulty in examining this type of sample for cysts. Methods are needed for detecting, identifying and enumerating cysts in soils and sediments. After suitable methods are developed and evaluated, they should be applied in laboratory and field studies to determine the persistence of *Giardia* in these media.

Additional information is also needed on the occurrence and survival of *Giardia* on surfaces and the potential for transmission by fomites. This information can assist in identifying and controlling risks of *Giardia* infection among children in day-care settings.

A variety of foods have been associated with giardiasis outbreaks, and it appears that the foods in these outbreaks were locally contaminated by infected food handlers. There are few quantitative data, however, on the occurrence of *Giardia* on various fruits, vegetables, and foods that are usually consumed without cooking. This may be related to the difficulty in recovering cysts from foods. Research is needed on methods to quantitatively detect these organisms and determine their survivability on foods. With the increased globalization of our food supply, more surveillance of domestic and imported foods should be conducted in order to develop data for use in risk assessments and to ensure against outbreaks. Recent outbreaks caused by another protozoan, *Cyclospora*, have been associated with contaminated fresh raspberries imported from Guatemala, and this problem underscores the need for additional knowledge about the occurrence and survival of *Giardia* and other protozoa on various foods.

Temperature has been demonstrated to be an important factor in *Giardia* survival. The effects of freezing have been determined in water and clinical specimens at low laboratory freezer temperatures. However, recent work has demonstrated that significant fractions of *Cryptosporidium* oocysts can survive temperatures just below freezing for relatively long time periods. Similar data, reflecting conditions likely to be encountered in the environment, should be developed for *Giardia*.

Information on occurrence of cysts in estuarine environments, and the factors in such environments that affect their survival, are limited and additional research should be conducted in this area.

The recent report that an isolate of *Flavobacterium* can kill *Giardia* prompted the suggestion that biological control of the organism may be possible. The identification of other organisms that could affect cyst survival may be fruitful and should be further explored.

H. Chapter IV. Health Effects in Animals

Since giardiasis is more common in immature animals and since growth retardation may be a consequence of this disease, improved diagnosis and treatment of animal giardiasis is a desirable goal. Incorrect diagnoses of *Giardia* infection may occur in animals when cysts are confused with yeasts or missed altogether in light infections. In this regard, it is important for veterinarians to be able to correctly process animal fecal specimens for microscopic examination.

Consideration should be given to the development of vaccines to prevent giardiasis in animals. The first successful *Giardia* vaccine, if one is developed, will probably be used in humans. When it is shown that such a vaccine is feasible, it should be a small step to develop similar vaccines for animals.

D. Chapter V. Health Effects in Humans

Many questions related to the host-parasite biology of *Giardia* remain. Following similar exposure, is the intestinal environment in persons who develop clinical giardiasis different from that of persons who do not? What is the role of adherence and invasion in determining the establishment and clinical course of *Giardia* infections? Do trophozoites mutate in vivo, and is this mutation in response to antigen-specific antibody? What are the functionally important

antigens? How are animal models relevant to understanding *Giardia* infections in man? Are intestinal phagocytic cells functionally active in the lumen of the intestine? How does *Giardia* cause illness, and can a vaccine be developed? Additional research is needed to help answer all of these questions.

It is questionable whether a vaccine can be developed, as this is already difficult enough for the blood dwelling parasites. Even if one can be developed, its use will likely be limited. Additional research should be conducted on the treatment of the disease. Although current drugs have been found effective, resistance has been observed for certain strains/genotypes. An alternative is to treat giardiasis with drugs aimed at the metabolism of *Giardia*. Research on its unique metabolism and differences with that of its warm-blooded hosts might be suggest a way to interfere with its life cycle.

Additional epidemiological studies are needed to better determine the prevalence of giardiasis and *Giardia* infection, sources of infection, quantitative estimates of risks associated with waterborne and other exposures, and the role of protective immunity. In order to conduct meaningful epidemiological studies, one of the highest priorities is the development of a sensitive and specific assay for anti-*Giardia* antibodies. The assay must be well characterized with information on the duration of serological response for each of the markers of interest. If an assay is available, population-based studies of endemic levels of *Giardia* infection could be done around the world. Serological studies can not only help in evaluating the efficacy of various water treatment systems in reducing risks but also in identifying other routes of infection.

There is variability in the humoral response to *Giardia* infection. Some patients with symptomatic infections fail to develop sufficiently high antibody levels for results to be called positive. In some patients, levels of anti-*Giardia* IgG antibodies remain elevated long after the infection appears to have been eradicated. No sero-diagnostic procedure has been reported that is capable of distinguishing asymptomatic from symptomatic infection, and additional work is

needed in this area. It is possible that existing sera from experimental studies can help evaluate some of the proposed Western blot serological assays. The specificity of antigen detection assays may be significantly improved by assays based on certain antigens groups (30/31 kDa, 57 kDa and high molecular weight antigens). Additional work is needed to evaluate these markers under both controlled and field conditions.

Additional research is needed on the suitability of using saliva for detection of anti-*Giardia* antibodies in patients with giardiasis. Saliva tests have an intuitive appeal since they can be applied to studies of children and do not require drawing blood.

E. Chapter VI. Risk Assessment

To improve risk assessments, better epidemiological information is needed about the risks of endemic waterborne giardiasis, role of immunity, and potential for secondary transmission among families where primary cases are waterborne. This will require clinical and specially designed epidemiological studies that include serological analyses as previously described. Additional research is needed to better describe the role of protective immunity in symptomatic illness and how long this might last. Information is also needed to better describe the risks of chronic diarrhea, malabsorption, and other chronic effects associated with exposure to *Giardia*.

Additional epidemiological studies are needed to provide better quantitative information about the endemic waterborne risks of *Giardia* infection and giardiasis for populations using unfiltered surface water, filtered surface water, and unfiltered groundwater sources. Risks from well designed epidemiological studies can then be compared with current estimates of risk from mathematical models used for risk assessment purposes. Epidemiologically determined risks can also be used to evaluate and revise, if necessary, the EPA's currently recommended annual risk

of *Giardia* infection that drinking water systems should attempt to maintain (one waterborne *Giardia* infection per 10,000 persons).

More complete information about exposures to *Giardia* cysts is needed to improve estimates from currently used models. This includes studies of the occurrence, transport, and fate of cysts in water sources, storm waters, reservoirs, animal waste ponds and lagoons, and septic tank effluents. Improved collection procedures and analytical methods are needed for detecting cysts in water and food samples as well as an inexpensive method to determine the viability of cysts detected. This will improve the exposure assessments used to estimate waterborne risk. Serological epidemiological studies can also assist with providing better exposure information for risk assessment purposes.

A major concern is the interpretation of the currently estimated waterborne risk of *Giardia* infection in the United States. The mathematical model used to estimate these risks is simple to use but has limitations. A recent model developed in the Netherlands considers several of these limitations and should be applied to water exposures in the United States. This will allow a comparison of risks with those estimated from the more simple model. Another recently developed risk assessment model considers population risks and attempts to include all of the relevant information that may be needed to estimate waterborne risks. This model should be used in combination with a sensitivity analysis to identify the parameters that may have the greatest effect on risk estimates. Waterborne *Giardia* infection risks should be estimated for the United States with each of the models.

F. Chapter VII. Analysis and Treatment

Combination analytical methods were developed for *Giardia* cysts and *Cryptosporidium* oocysts in the same sample because early studies suggested that acceptable recoveries of both could be obtained. Performance evaluation studies have shown that the methods generally have

low recovery and poor precision. Both recovery and precision are better for *Giardia* than for *Cryptosporidium* and consideration should be given to developing methods specific to each. A draft method recently proposed by the EPA (Method 1622) is recommended only for oocysts at the present time. The method protocol includes 10 L sample volumes, cartridge membrane filtration, immunomagnetic separation and microscopic examination with or without flow cytometric cell sorting. Work is in progress to apply this methodology to *Giardia* detection. More research needs to be done on practical environmental sample methods for determining the species of cyst detected and whether or not they are viable or infective. Research is needed on the appropriate sample volumes for raw and treated waters and on whether substituting 10 L sample volumes for 100 L will result in improved method recovery and precision. Also, the effect of collecting larger sample volumes with methods designed for smaller volumes should be evaluated.

The availability of protocols or guidelines listing minimum requirements for comparing different procedures or methods for cyst detection could assist investigators in producing the required data for demonstrating acceptability or equivalency of modifications to approved methods. Consideration should also be given to developing a mechanism for analyst and laboratory approval processes that might be established to continue the certification program initiated with the ICR.

With the recent increased emphasis on studies of the effectiveness of water disinfection and filtration to inactivate and remove *Cryptosporidium* oocysts, less attention has been paid to *Giardia*. The assumption is made that if a disinfectant is sufficient to inactivate *Cryptosporidium* that it will also be effective for *Giardia* . A similar reasoning is applied to water filtration technologies. A more critical approach should be taken because these two protozoa have different life cycles and biology. It may not be appropriate to assume that all disinfection and filtration effectiveness studies conducted for *Cryptosporidium* will be effective for *Giardia*. Whether a technology is equally effective will depend on the mechanism of

inactivation and mechanism of filtration. For example, if the mechanism for removal is primarily physical straining, as in slow-sand, diatomaceous earth, or membrane filtration technologies, then *Giardia* cysts should be removed at least as effectively as *Cryptosporidium* oocysts, since the cysts are larger. However, in conventional and direct granular filtration the optimum operating conditions for removal of *Cryptosporidium* oocysts may not be the same as for *Giardia* cyst removal. Additional research is needed to better define optimum operating parameters and coagulant chemicals that are effective for the simultaneous removal of both cysts and oocysts by these filtration processes. In regard to disinfection, research is needed on the inactivation of *Giardia* cysts by the newer UV processes and other electrotechnologies since these technologies are now being evaluated for *Cryptosporidium*. Also, the potential for photoreactivation of UV inactivated cysts should be examined as well as the potential for damage repair when chemical disinfectants are used.

Since the information on effectiveness of chemical disinfectants is based on results of laboratory studies in demand-free water, additional studies should be conducted to compare the effectiveness of disinfectants under representative conditions in natural waters.

In response to the recent report that an isolate of *Flavobacterium* can kill *Giardia*, research should be encouraged on the development and application of biological control agents for wastewater and drinking water treatment.

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