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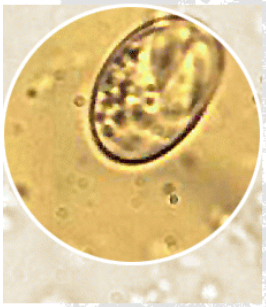
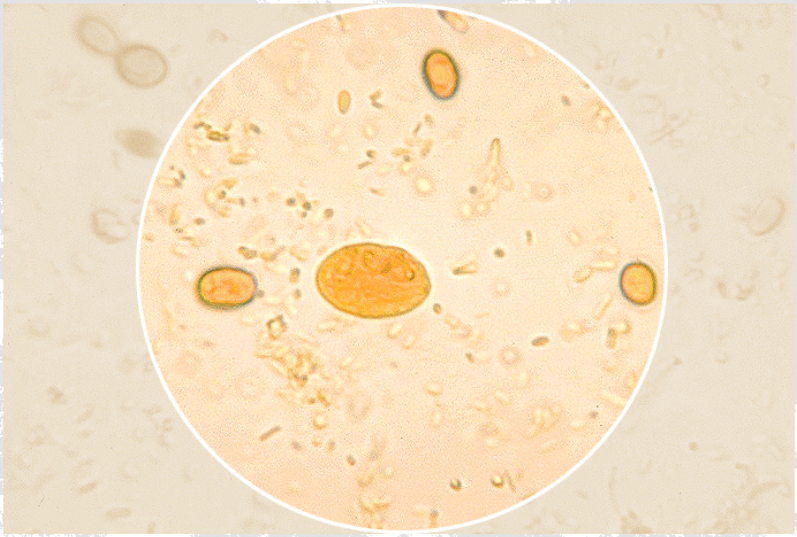
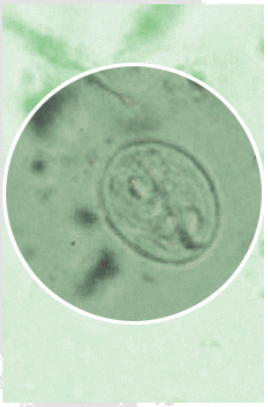
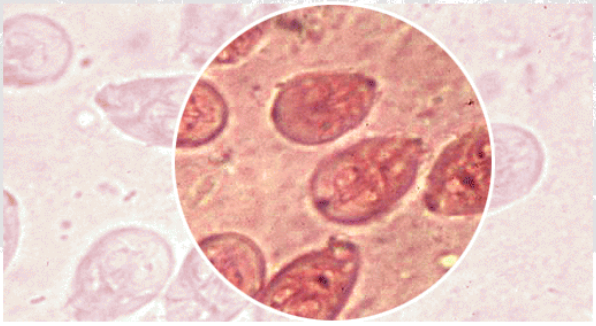
Giardia: Drinking Water Health Advisory









I. INTRODUCTION

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

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

This HA is based on information presented in the OW's Criteria Document (CD) for *Giardia*. Persons desiring further information should consult the CD. This document will be available from the U.S. Environmental Protection Agency, OW Resource Center, Room M6099; Mail Code: PC-4100, 401 M Street, S.W., Washington, D.C. 20460; the telephone number is (202) 260-7786. The document can also be obtained by calling the Safe Drinking Water Hotline at 1-800-426-4791.

II. GENERAL INFORMATION

Members of the genus *Giardia* are binucleate, flagellated protozoan parasites which belong 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. *Giardia* cause infection by attaching to the wall of the small intestine in the upper gastrointestinal tract of humans and other vertebrates. *Giardia* have been reported in a variety of mammals and in lower vertebrates. *Giardia* responsible for human infections will be found referred to variously as *G. duodenalis*, *G. intestinalis*, or *G. lamblia*.

Organism Description

While numerous species of *Giardia* have been described, there is no general agreement on the criteria which define species in this genus. Criteria that has been used 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 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. 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. 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.

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 it is ingested. Following ingestion of a viable cyst, the excystation process to a trophozoite is initiated by low pH conditions in the stomach. Excystation is completed in 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 in the upper bowel and continues in the lower small intestine where the trophozoite rounds up and secretes cyst wall components. The wall provides protection when cysts pass out of the host with the feces. Unlike the trophozoites, cysts are not motile.

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. 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 in cysts were less compact than those observed in the trophozoite (Friend, 1966), possibly accounting for the apparent absence of median bodies in cysts when viewed with visible-light microscopy. Gillin et al. (1989) reported that in a Type I cyst the median body is visible when viewed in relief with Nomarski differential interference contrast optics.

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 may remain viable in water for long periods of time under typical environmental conditions. Survival in water is dependent primarily on decreased water temperature; no relationship was found between cyst survival and water pH, dissolved oxygen, turbidity, color, hardness, ammonia, nitrate or phosphorous. After being stored in water for 77 days at 8 C, *G. lamblia* cysts were found to be viable by dye exclusion testing (Bingham et al., 1979). Cysts survived about 26 days at 21 C and about 6 days at 37 C. Less than 1% of cysts survived freezing at -13 C for 14 days. Raising the temperature to boiling immediately inactivated the cysts. *G. muris* cysts were found to be infective in mice after periods of 28 days storage in distilled water at 5 to 7 C; none were found infective after 56 days (deRegnier et al., 1989). *G. muris* cysts remained viable for 2 to 3 months when stored in natural surface water at temperatures of less than 10 C. The thermal death point (i.e., the lowest temperature at which *G. muris* cysts are inactivated in 10 minutes) is 54 C (Schaefer et al., 1984).

In marine waters in Hawaii, Johnson et al. (1997) found that the viability of *G. muris* cysts, as determined by excystation, was reduced by 99.9% in 3 hours during sunlight and 77 hours in conditions of darkness.

Using dye exclusion as an indicator of viability, Deng and Cliver (1992) found that a 90% reduction of viable *G. lamblia* cysts occurred in septic tank effluent within 18 days. In anaerobic digester sludge from municipal wastewater treatment plants, Van Praagh et al. (1993) found that 99.9% of *G. muris* cysts were inactivated in 15.1 days, 20.5 hours, and 10.7 minutes at temperatures of 21.5 , 37 , and 50 C, respectively.

Direct Transmission Between Humans

Giardia cysts are highly infective for humans (Rendtorff, 1954a, b; 1979). In a controlled, clinical study of male volunteers who were fed human-source *Giardia* cysts contained in gelatin capsules, a dosage of ten cysts produced human infection. Infection was assessed by observing presence of *Giardia* in fecal smears. Eight dosage levels ranging from 1 to 1,000,000 cysts per capsule were studied.

Rendtorff (1954a, b;1979) found that the incubation period of giardiasis in human volunteers ranged from 9 to 22 days with a mean of 13.1 days. Benenson (1995) reported 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 was typically 12 to 19 days. In human volunteers, Nash et al. (1987) found that diarrhea or loose stools appeared within 7.25 (± 2.99) days of inoculation with *G. lamblia* trophozoites by intestinal intubation.

Nash et al.(1987) found evidence of possible strain differences between *Giardia* trophozoites that had been isolated from two infected persons. All five volunteers that were inoculated with 50,000 trophozoites of an isolate from one of the human hosts became infected, but none of five volunteers that received 50,000 trophozoites of an isolate from the other host became infected.

Cross-species Transmission of *Giardia*

Giardia from some animals exhibit an apparent high degree of host specificity; other isolates may infect more than one host. Recently conducted, carefully controlled studies indicate that cross-species transmission of *Giardia* can occur.

Transmission Between Animals. Studies using *Giardia*- free mice showed that *G. simoni, G. muris* and *G. peromysci* were host-specific while *G. microti* and *G. mesocricetus* were not. Mice were 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. Erlandsen et al. (1988a) found that beavers did not become infected when inoculated with cysts of *G. ondatrae* from muskrats or with *G. muris* from mice; however, five of eight (62%) muskrats became infected when administered *Giardia* cysts of beaver origin.

Transmission Between Animals and Humans. Erlandsen et al.(1988b) showed that viable cysts, from symptomatic human donors, can cause infection in the beaver and muskrat and concluded that the beaver and muskrat were possible intermediate reservoirs for *Giardia* that infect humans. Experimental studies suggest that rats, mice, dogs, cats, gerbils, and mule deer are also capable of harboring *Giardia* that may also infect humans. The importance of animals as a source or reservoir of human infection, however, remains controversial. Of all of these animals, evidence suggests that the beaver and possibly the muskrat may serve as important reservoirs of infection. The argument supporting the importance of these aquatic animals is based primarily on the frequency with which giardiasis in North America is transmitted by contaminated water. 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. The beaver has also been implicated as a source of contamination in waterborne outbreaks (Craun, 1990; Isaac-Renton, 1994). Both of these 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 found in humans, has been identified in the muskrat (van Keulen et al., 1998). It is possible that the beaver and the muskrat harbor two types of *Giardia*.

One type may be highly adapted to the animal and is rarely, if ever, transmitted to humans. The other type, which may be acquired by the animal from human sources, may multiply in the beaver or muskrat and in turn be transmitted via water back to humans.

Environmental Occurrence of *Giardia*

The limitations of the methods used to detect and quantify the cysts should be kept in mind when evaluating the significance of environmental occurrence data. Quantitative data may not be reliable due to the relatively low efficiency and precision of sample collection procedures and analytical detection methods. In addition, no information on viability, infectivity, or species identification is generally provided when *Giardia* cysts are detected in environmental samples.

Waste water. Examination of *Giardia* in raw sewage has been suggested as a way to assess the prevalence of *Giardia* infection and detecting possible outbreaks in communities. Only one 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 cities in the United States (Jakubowski et al., 1991).

Sykora et al. (1991) found a seasonal distribution of *Giardia* ranging from 4 to 14,000 cysts/L in raw sewage samples collected monthly from 11 U.S. cities. Cyst levels were highest during late summer through early winter. The geometric mean level of *Giardia* at each site ranged from 642 cysts/L at a Pennsylvania sewage treatment plant to 3,375 cysts/L at a California plant. All of the raw sewage samples were positive for *Giardia*, 48% of secondary effluent samples contained from 1 to 44 cysts/L, and 80% of the sludge samples contained from 70 to 30,000 cysts/L. These analyses used the iodine staining method which LeChevallier et al. (1990) has found will yield significantly lower numbers of cysts than an immunofluorescence assay (IFA).

Enriquez et al. (1995) used IFA to examine 130 samples from three wastewater plants over a three year period. A geometric mean of 8.3 and 6.6 cysts/40 L was found in secondary effluents from each of two activated sludge plants; a geometric mean of 3 cysts/40 L was found in the tertiary effluent of water reclamation plant. No seasonal variation in cyst levels was observed. Mayer and Palmer (1996) reported levels of 13,000 and 11 cysts/L in influent and secondary effluent samples, respectively, from a large metropolitan wastewater plant in California. At a Maryland wastewater plant, Casson et al. (1990) found a geometric mean of 4 and 11 cysts/L in activated sludge and trickling filter effluent samples, respectively; a geometric mean of 137 cysts/L was found in the plant influent.

Roach et al. (1993) found *Giardia* in 44 samples of raw sewage from five locations in the Yukon at levels ranging from 26 to 3,022 cysts/L. In five samples of treated sewage from two locations, levels ranged from 2 to 3,511 cysts/L. Robertson et al. (1995) found that all raw sewage samples collected bimonthly for one year from six sewage treatment plants in Scotland were positive for *Giardia* at levels ranging from 102 to 43,907 cysts/L. Hirata and Hashimoto (1997) found 95% of the samples from nine activated sludge sewage treatment plants in the Kanto area of Japan positive for cysts by IFA. *Giardia* were found in all of the raw sewage samples (geometric mean = 1,500/L; range = 130 to 7,900/L), 86% of the secondary effluent samples (geometric mean = 18; range = 2 to 310), and 82% of the final effluent samples (geometric mean = 14/L; range, 4 to 130/L).

Grimason et al. (1996) found *Giardia* cysts in 37% of raw sewage samples (range = 1,000 to 25,000 cysts/L) from a stabilization pond in Kenya and in 100% of similar samples (range = 230 to 25,000 cysts/L)

from a stabilization pond in southern France. Cysts were detected in all samples of the pond effluent in Kenya but in only 44% of those at the French plant. Wiandt et al. (1995) found levels of 0.1 to 2.5 cysts/L in the French effluent during the wintertime but did not detect cysts during the spring and summer.

Surface Water. Cysts have been found all months of the year in surface waters from the Arctic to the tropics in even the most pristine of surface waters. Occasionally, seasonal variations are reported. Cyst levels are generally higher in rivers or streams influenced by agricultural (e.g., cattle or dairy farming) or residential (e.g., sewage discharges) activities. Cysts occur in surface waters throughout the year. In North America, levels are generally higher in the late summer to early winter. Generally, no relationship is seen between cyst levels and bacterial indicators of water contamination. In the United States, levels of *Giardia* in water are somewhat lower than *Cryptosporidium*; in Canada, surveys have found higher levels of *Giardia* than *Cryptosporidium*.

It should not be assumed that cyst levels remain constant in surface waters. Levels may fluctuate significantly due to weather events, agricultural practices and wastewater treatment plant type and operational practices. In two California watersheds, peak levels of *Giardia* occurred following storm events, especially the first storm of the season, and the first-flush run-off from storm events significantly affected cyst occurrence and levels in source waters (Stewart et al., 1997). First-flush samples most often detected *Giardia* (60%) and at the highest levels (range = 25 to 16,666 cysts/100 L). Only 19% of the grab samples detected *Giardia* cysts (range = 42 to 2,428 cysts/100 L).

Cyst levels in drinking water also vary depending on source water characteristics and water treatment practices. In 26 drinking water outbreaks in the United States, levels of *Giardia* cysts ranging from <1/100L to 580,000/100L have been detected from tap water, treated water, or unfiltered water sources.

Hibler (1988) analyzed 4,423 water samples from 301 municipalities in 28 states using a zinc sulfate flotation/iodine staining method to detect *Giardia* cysts. Cysts were detected in 26% of source water samples and 11% of finished water samples. Positive samples were found in 28% of creeks, 26% of rivers, and 10% of lakes used for water sources. Rose et al. (1991a) detected *Giardia* cysts in surface water sources but not in drinking water; 16% of 257 water samples from 17 states were positive. Levels of *Giardia* cysts were not correlated with turbidity or total or fecal coliforms. Samples collected in the fall were more likely to be positive, and cyst levels tended to be higher in summer/fall samples. The geometric mean levels found in polluted and pristine rivers were 11 cysts/100 L (maximum = 625/100 L) and 0.35 cysts/100 L (maximum = 12/100 L), respectively. 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.

LeChevallier et al. (1991a) detected *Giardia* cysts in 17% and 81% of the drinking water and raw water samples, respectively, from 66 surface water treatment plants in 14 states and one Canadian Province. Only 13% of *Giardia* cysts observed in these water samples had morphological characteristics suggesting that the cysts may be viable. There was a greater probability of finding high levels of *Giardia* as levels of bacterial indicators and turbidities increased. Levels in raw water ranged from 4 to 6,600 cysts/100 L (geometric mean = 277 cysts/100 L). In a subsequent survey, LeChevallier and Norton (1995) detected *Giardia* cysts in 45% of raw water samples, ranging from 2 to 4,380 cysts/100 L (geometric mean = 200 cysts/100 L). *Giardia* cysts were detected in 5% of filtered plant effluent samples at levels ranging from 0.98 to 9 cysts/100 L.

Norton et al. (1995) found *Giardia* in 23% of 147 samples from 15 sites in New Jersey source waters; the mean level in positive samples was 210 cysts/100 L (range = 40 to 630/100 L). In Wisconsin, Archer et al.

(1995) found that all 18 streams sampled were positive for *Giardia*; the highest level was 2,601 cysts/100 L from a stream characterized as pristine. Cysts were found in 5% of the samples from drinking water intakes at levels up to 125 cysts/100 L (median = 1.2/100 L).

A survey of surface water treatment plants in Pennsylvania found *Giardia* in 23% of 148 raw water samples at levels ranging from 0.4 to 5.7 cysts/100 L (Consonery et al., 1997). No cysts were detected in finished water samples. LeChevallier et al. (1997) found 13% of the influent samples from six open finished water reservoirs in New Jersey positive for *Giardia* at a geometric mean level of 1.9 cysts/100 L; 15% of the reservoir effluent samples were positive at a geometric mean of 6.1 cysts/100 L.

States et al. (1997) found that over a two-year period 63% and 54% of samples from the Allegheny and Youghiogheny Rivers near Pittsburgh were positive with geometric mean levels of 34 and 118 cysts/100 L, respectively. Sources of cysts in the Allegheny River were identified as combined sewer overflows, a dairy farm, and a sewage treatment plant. Filter backwash samples from the drinking water treatment plant were positive 8% of the time at levels of 15 to 237 cysts/100 L (geometric mean = 59/100 L). Okun et al. (1997) summarized *Giardia* monitoring results for three of New York City's drinking water reservoirs. *Giardia* was found in 36%, 29% and 46% of samples and at mean levels of 1.2 cysts/100 L (maximum = 9.3/100 L), 0.7 cysts/100 L (maximum = 8.2/100 L) and 1.3 cysts/100 L (maximum = 23.4/100 L) in the Catskill, Delaware and Malcolm Brooks reservoirs, respectively.

In a biweekly survey for a one-year period, Rose et al. (1988) detected *Giardia* cysts in 31% of samples from a lake contaminated by sewage effluents and cattle pastures. Higher levels were found in a river just downstream from the cattle pastures; the geometric mean was 22 cysts/100 L (maximum = 625 cysts/100 L). The geometric mean level detected in the lake was 8 cysts/100 L (maximum = 222 cysts/100 L). No relationship was found between cyst levels and either total or fecal coliforms or turbidity. In three pristine watersheds in Washington, Ongerth (1989) found assessed *Giardia* cysts in 43% of 222 samples during a nine-month period. Cyst levels ranged from 10 to 520 cysts/100 L. The median levels in the Green, Cedar, and Tolt Rivers were 6, 4, and 0.3 cysts/100 L, respectively. In two relatively pristine watershed in the Olympic Mountains, Ongerth et al. (1995) found *Giardia* at levels from 0.2 to 3 cysts/100 L; higher levels were found in the more heavily used watershed.

Wallis et al. (1996) detected *Giardia* cysts in 21% of 1,760 raw and 18% of treated water samples from 72 Canadian cities, 58 of which treated their drinking water by chlorination alone. Most water samples contained fewer than 2 cysts/100 L. The highest level, 230 cysts/100 L, was found in a community experiencing an outbreak of waterborne giardiasis. *Giardia* cysts were recovered more frequently and in higher levels in late winter-early spring and were more common than *Cryptosporidium* oocysts. Cysts infective for gerbils were detected in 2% of raw water samples and in 8% of treated water samples.

Chauret et al. (1995) found *Giardia* cysts in 78% of 41 raw water samples (range = 1 to 52 cysts/100 L) collected during the summer months at 15 sites along the Ottawa and Rideau Rivers. Both watersheds were characterized as relatively pristine. At the intakes of two drinking water treatment plants located on the Ottawa River, *Giardia* was found in 83% of raw water samples at levels up to 25 cysts/100 L; no treated water samples were positive. No relationship was found between the presence of *Giardia* and any of the following: fecal and total coliforms, fecal streptococci, *Aeromonas* sp., *Pseudomonas aeruginosa, Clostridium perfringens*, algae and coliphages. Isaac-Renton et al.(1996) surveyed 86 sites in British Columbia, none of which were downstream of large urban sewage discharges. All watersheds were open to public access and many had

agricultural activities in the vicinity. Of 153 raw water samples, 64% were positive for *Giardia* at geometric mean levels of 2.9 cysts/100 L. No seasonal variation was observed. Of 91 chlorinated water samples, 59% were positive at a geometric mean level of 2.1 cysts/100 L; 34% of the positive samples were found to be infective for gerbils.

Roach et al. (1993) found *Giardia* cysts in 32% of samples collected from pristine streams in the Canadian North. A sample taken through the ice at Lake Laberge contained 7.5 cysts/100 L, the highest level found in the study. No cysts were found at the drinking water intake at Dawson, but cysts were found in 17% of the samples from the Whitehorse intake (0.2 to 1.4 cysts/100 L). Payment and Franco (1993) found *Giardia* in 94% of the raw water samples from three drinking water treatment plants in the Montreal area at levels up to 2,800 cysts/100 L; one finished water sample was positive (0.7 cysts/100 L). Isaac-Renton et al. (1996) studied two Canadian water districts over a 2-year period. In the Black Mountain Irrigation District, all raw water samples were positive at levels ranging from 7 to 2,215 cysts/100 L (geometric mean = 229 cysts/100 L). At the water disinfection plant, 77% of chlorinated water samples were positive with levels ranging from 0.3 to 371 cysts/100 L (geometric mean = 31 cysts/100 L). In addition, 71% of tap water samples were positive at levels from 1.5 to 18.5 cysts/100 L; 11% of positive samples were infective for gerbils. A seasonal trend was noted with peak levels occurring in late autumn and early winter. In the Vernon Irrigation District, all raw water samples were also positive, but cyst levels were lower (range = 8 to 114 cysts/100 L; geometric mean = 30/100 L). Of the chlorinated water samples, 98% were positive containing 2 to 73 cysts/100 L (geometric mean = 14/100 L). No clear seasonal trend was observed and none of 66 positive samples were found to be infective.

At a water plant in Germany, Karanis et al. (1996) found 83% of raw water samples positive for *Giardia* at levels of 2 to 103 cysts/100 L (median = 24.5); 84% of backwash water samples were positive at levels ranging from 3 to 374 cysts/100 L. At six surface water treatment plants in Germany, Karanis et al (1998) found 64% of the raw water samples positive for *Giardia* at an average level of 88.2 cysts/100 L (maximum = 1,314/100 L). About 15% of the drinking water samples were positive, and a sample of backwash water from an activated carbon filter was found to contain 3,428 cysts/100 L. In Selangor, Malaysia, Ahmad et al. (1997) found that 90% of the raw water samples from two drinking water plants were positive for *Giardia* at levels of 100 to 2,140 cysts/100 L. No *Giardia* cysts were detected in the treated water samples, and no relationship was found between cyst levels in the raw water and fecal coliforms or physical parameters. In two Hong Kong rivers, Ho and Tam (1998) found high levels of *Giardia* cysts. Up to 46,880 cysts/100 L were found in one river and more than 10,000 cysts in the other. The highest cyst levels were found at sampling sites near the more densely populated areas, but no relationship was found between levels of cysts and *E. coli*.

Groundwaters. Archer et al. (1995) did not detect *Giardia* in any of 17 samples from six wells in Wisconsin. Hibler (1988) found *Giardia* cysts in 19% of springs and 3% of wells sampled. Lee (1993) reported the contamination of two wells in Pennsylvania by surface streams less than 100 feet from the wells;*Giardia* was recovered from all samples collected from the wells. Hancock et al. (1997) collected 463 groundwater samples from 199 sites in 23 states in the United States; *Giardia* cysts were found in 14% of the springs, 1% of the vertical wells, 36% of the horizontal wells, and 25% of the infiltration galleries. The mean levels in positive water samples was 8 cysts/100 L (range = 0.1 to 120/100 L).

Cisterns. Over a one-year sampling period in the Virgin Islands, Crabtree et al. (1996) found that 26% of 45 samples from cisterns were positive for *Giardia* cysts. The reported mean level was 1.09 cysts/100 L (range = <1 to 3.79 cysts/100 L). In January, 18% of the cisterns were positive for *Giardia*; in July, 54% were positive.

Soil. The wide distribution of cysts in human and animal populations suggests that soil may be contaminated with *Giardia* through fecal deposition, irrigation and sewage treatment practices. However, no published data are available on cyst levels in soil, survival in soil, or transport through soil matrices. A progress report from the Cornell University Whole Farm Planning Scientific Support Group (1993) discussed development and evaluation of a soil sampling protocol and detection method to evaluate the prevalence and transport of *Cryptosporidium* oocysts and *Giardia* cysts on dairy farms within the New York City watershed.

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

Surfaces. Cody et al. (1994) evaluated a method for detecting *Giardia* cysts from environmental surfaces, and 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 to 190 cysts on a surface area of 50 cm^2 or with 10 to 20 cysts/400 cm^2. Recoveries from stainless steel surfaces were lower and false negatives were higher. Cysts were not recovered from wood and fiberglass surfaces spiked with 190 cysts/400 cm^2. In two day-care centers; two fiberglass chairs (6%) and one Formica® table (2%) surface were found to be positive for *Giardia* cysts.

Food. There are a lack of quantitative data on the occurrence of *Giardia* cysts in foods, and improvements are needed in both sampling and analysis. Although foodborne giardiasis outbreaks in the United States have involved fish, sandwiches, vegetables, fruit and noodle salad, no information was available for *Giardia* levels in these foods. Barnard and Jackson (1984) described four techniques that were originally developed for clinical specimens and had been adapted to foods. One of the techniques (sedimentation/zinc sulfate flotation) was used by Italian investigators to isolate *Giardia* cysts from lettuce in 1968; 75% of 64 heads of lettuce collected at random from four markets in Rome, Italy, were found positive for cysts. Oliveira and Germano (1992) found *Giardia* sp. in 4% of lettuce and 10% endive sampled from vegetables in S. Paulo, Brazil. Bier (1991) found that a method employed by the Food and Drug Administration had poor recovery of spiked *Giardia* cysts from fruits and vegetables. Fayer et al. (1998) examined 360 oysters (*Crassostrea virginica*) collected from six sites in the Chesapeake Bay. *Giardia* cysts were not found in any of the samples but were detected in positive control specimens.

III. HEALTH EFFECTS

Giardia is transmitted via the fecal-oral route of exposure, and both endemic and epidemic transmission may occur. Human and animal effects are discussed.

Health Effects in Humans

Giardiasis can present as (1) asymptomatic infection; (2) acute diarrhea; (3) chronic diarrhea. In addition to diarrhea, symptoms may include steatorrhea, abdominal cramps, bloating, flatulence, weight loss, and vomiting. Stools may be pale, greasy, and malodorous. Malabsorption of fats or fat-soluble vitamins may also occur. When daily losses of fat in feces are greater than 7 grams, the condition is classified as steatorrhea (Hall, 1994). Weight loss can be significant under these circumstances with a loss of 10 to 20% of usual or

ideal body weight (Farthing, 1996). Giardiasis may result in growth impairment in young children or other complications, but rarely does it cause death.

Giardia infection is often asymptomatic for children. It is not clear whether the initial infection is always acquired without producing symptoms, since infection may result in a transient, mild, diarrheal illness that can pass without notice.

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. Chronic giardiasis appears to be infrequent, but when it occurs, may persist for years, sometimes with asymptomatic periods. 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).

Generally, patients resolve their infections spontaneously, but patients with acquired immune deficiency syndrome (AIDS) may have more serious and prolonged infection.

The mechanisms by which *Giardia* produces diarrhea and malabsorption are not well understood, and the immunologic determinants for clearance of acute infection and development of protective immunity are not well defined. Differences in virulence, pathogenicity, infectivity, growth, drug sensitivity, and antigenicity have been reported. The epidemiology of giardiasis is also complicated by an apparent genetic heterogeneity in this species. In endemic areas where extensive heterogeneity exists, mixed infections with more than one genotype may occur.

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). Hospitalized cases were primarily children under the age of five; volume depletion or dehydration (22%) was the most frequently listed co-diagnosis on admission. In Michigan, 66% of the hospitalized cases of giardiasis in young children were one year of age or younger. The high hospitalization rate for young children with giardiasis in the United States may reflect the physician's concern about a possible adverse outcome rather than the severity of their illness. Young children have fewer reserves and are more susceptible to fluid and nutritional losses from infection.

Case reports and epidemiological studies suggest several conditions or complications that may be associated with *Giardia* infection, however, the evidence for a causal role of *Giardia* is limited. The most often reported and a potentially serious consequence of giardiasis is nutritional insufficiency and its consequences. In adults this rarely produces serious sequelae, especially if the infection is treated promptly or spontaneous remission occurs, but in infants and young children, nutritional insufficiency can have profound effects on growth and development (Farthing, 1996).

Although the significance of *Giardia* as a cause of growth retardation is debated, weight loss and malabsorption have been described in children infected with *Giardia* and some children have suffered from impaired growth. Almost 19% of the young children who were hospitalized for giardiasis in Michigan were diagnosed with failure to thrive (a concern that growth is slower than expected). In Scotland, 11% of children who were hospitalized for giardiasis were also lacking in expected normal physiological development (Robertson, 1996). The significance of impaired growth and development associated with giardiasis will differ among children in developed and developing countries, depending on capabilities for diagnosis and medical treatment and on other conditions (e.g., socioeconomic status) which may affect the ability of the child to catch-

up in growth and complete pubertal development. Catch-up growth and completion of pubertal development is possible if nutrition is adequate.

In some (Filer, 1996) but not all studies (De Morais et al., 1996), malabsorption of iron has been associated with symptomatic giardiasis in some children but not in others. Allergic reactions have also been reported (Di Prisco et al., 1998). Inflamation of the synovial membranes of major joints has been observed in some children with giardiasis (Letts et al., 1998); following anti-giardial chemotherapy, intestinal and synovial symptoms were abated. An association between symptomatic giardiasis and pancreatic or hepatic disease is suspected, but this association has not been carefully studied. Several case reports have seen these disorders in adults with giardiasis (Carroccio et al., 1997; Nakano et al., 1995; Roberts et al., 1988). Roberts et al. (1988) suggested that if such an association exists, the relatively common occurrence of pancreatic insufficiency or hepatic cirrhosis in persons with cystic fibrosis might predispose them to infection with *Giardia*. Although additional studies are needed to clarify a possible association between cystic fibrosis and *Giardia* infection, the clinical implications of *Giardia* infection (i.e., malabsorption of fat and fat-soluble vitamins) in cystic fibrosis patients, especially children should be recognized. Corsi et al. (1998) suggest that asymptomatic, non-progressive "salt and pepper" retinal changes found in a study in Italy may be relatively common in young children with giardiasis. These lesions were not felt to cause functional changes in the retina, but this finding should be confirmed in longer term follow-up studies. Increased risk of these lesions may reflect a genetic predisposition.

Treatment. As with all diarrheas, fluid replacement is an important aspect of treatment. Anti-giardial drugs are also important in the management of individual cases of giardiasis, but they may not prevent frequent reinfection of children who attend day-care centers or live in communities where *Giardia* exposures are routine. Chemotherapeutic agents used for treatment of giardiasis include metronidazole, tinidazole, quinacrine, furazolidone, albendazole, and ornidazole (Benenson, 1995; Farthing, 1996; Adam, 1991; Ortega and Adam, 1997; Rabbani and Islam, 1994). Various doses and treatment periods are recommended for each drug. These agents have different effectiveness in their ability to clear *Giardia*, and side-effects should be considered. Drug resistance or re-infection may occur. Paromomycin is recommended for pregnant women, but the cure rate may be low.

Prevalence. 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. 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). *Giardia* infection tends to be more common in children than adults.

National surveys of stool specimens submitted for parasitological evaluation found that *G. lamblia* is the most frequently identified parasite in the United States. *Giardia* was found in 7.2% of 216,675 specimens examined in 1987 and 5.6% of 178,786 specimens examined in 1991 (Kappus et al., 1994). Depending on the age group and country, the prevalence of infection among children world-wide has been found to range from 1% to 36% and may be as high as 68%. In many developing countries, *Giardia* infections are acquired in early childhood, and by five years of age, most children have been infected at least once (Farthing, 1986). This high prevalence is likely due to frequent opportunities for exposure. In the United States, the highest incidence and prevalence of infection is found among children under 5 years of age, especially for children attending day-care centers. In two counties of Washington State, 7.1% of children aged 1 to 3 years were found to be infected

(Harter et al., 1982). Bartlett et al. (1991) found that 11% of infants and toddlers tested for admission to day-care centers in Arizona were already infected. In nine outpatient clinics in the United States, *Giardia* was detected in 15% of children (age 2-11 years) with acute diarrhea (Caeiro et al., 1999). In Vermont, children aged one to four years had the highest incidence rate for symptomatic *Giardia* infection of any age group (Birkhead and Vogt, 1989). In British Columbia, the majority of *Giardia*-positive patients were in the 1-5 year age group (Isaac-Renton and Philion, 1992).

Sensitive Populations. Immunocompromised persons, especially patients with AIDS and achlorhydria may be more susceptible to symptomatic infection (Benenson, 1995). 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, 18% of 126 homosexual males were found to be positive for *Giardia* compared to a 2% positivity among 5,885 other patients. Farthing (1996) reports that giardiasis is one of the few potentially treatable causes of diarrhea in persons with AIDS and that chronic giardiasis does not appear to be a major clinical problem in persons with HIV infection or AIDS patients.

Immunity. Most persons 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. Responses to a number of different *Giardia* antigens have been reported, but it is uncertain which, if any, of these responses predict a reduced risk of either infection or illness. A serological investigation of a waterborne outbreak in Vermont suggests that elevated IgA antibody to *G. lamblia* may effectively determine exposure to cyst contaminated water and subsequent illness during waterborne outbreaks (Birkhead et al., 1989).

Development of protective immunity to *Giardia* is considered a relative lengthy process and does not necessarily develop following a single infection. Only partial protective immunity to illness from *Giardia* infection is likely to develop. In a community that experienced two waterborne giardiasis outbreaks separated by a five year period, persons infected during the first outbreak were at significantly lower risk during the second outbreak (Isaac-Renton et al., 1994).

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. Infants and young children may have increased susceptibility to giardiasis because they lack protective immunity and have increased opportunities for exposure (Robertson, 1996).

Giardia is not transmitted from mother to fetus, but infants can acquire infections at an early age suggesting that mothers may infect their children very soon after childbirth. Studies in developing countries have provided evidence that breast-fed infants have a lower risk of *Giardia* infection than non-breast-fed infants, but several studies have also reported similar risks of infection or diarrhea in breast-fed and non-breast-fed infants. Increased risks for some breast-fed infants in developing countries may be due to high levels of exposure from siblings or from home and environmental sources. Breast milk may protect some infants from *Giardia* infection because of protective immunity of secretory antibodies in breast milk or breast milk enzymes

which have been found *in vitro* to release substances that kill *Giardia* trophozoites. The use of breast milk also provides fewer opportunities for the infant to become infected from other sources, such as water.

Health Effects in Animals

Organisms in the genus *Giardia* have been reported as intestinal inhabitants within a variety of mammals, birds, reptiles, amphibians, and fishes. *Giardia* infection in animals is also spread via the fecal-oral route. Infection occurs worldwide, in most animal species; is more often than not asymptomatic; and is much more likely to spread within a host species than from one host species to another (Erlandsen et al., 1988b). In animal species (e.g., calves, cats, dogs) whose *Giardia* infections have been studied in detail, the resultant effects resemble those seen in humans. *Giardia* infection is more likely to be symptomatic in young animals. Mortality appears to be significant in some animals, e.g., chinchillas and budgerigars.

Treatment. Symptomatic infection in animals that require therapy usually respond to the same agents, with the same caveats, used in treating human infections. Albendazole and fenbendazole, have been shown to clear *Giardia* cysts from infected dogs and calves (Barr et al., 1993, 1994; Zajac et al., 1998; Xiao et al., 1996; O'Handley et al., 1997). Metronidazole or furazolidone has been used for *Giardia* infections in cats (Kirkpatrick, 1986; Patton 1998). *Giardia* infection in horses has been successfully treated with metronidazole, and birds have been treated with fenbendazole (Patton, 1998). Albendazole is suspected of being teratogenic and should not be given to pregnant animals. Olson et al. (1997c) found that immunization of puppies with a trophozoite-derived *Giardia* vaccine provided protection against giardiasis.

Prevalence. *Giardia* is a common protozoan parasite of farm animals. Buret et al. (1990) and Olson et al. (1997a) found 18 to 38% of sheep, 10 to 29% of cattle, 20% of horses, an 9% of pigs were infected with *Giardia*; a higher prevalence was found in lambs (36%) and calves (28%). 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). The prevalence of *Giardia* infection in beaver was found to be 7% to 16% in different parts of the United States; in muskrats the prevalence was greater than 95% (Erlandsen et al., 1990b). A high prevalence of *Giardia* (>90%) has been reported for wading birds (egrets and herons) (Erlandsen, 1994; Erlandsen et al., 1990a, b). *Giardia* have also been found in voles, mice, shrews, native marsupials, Australian bushtail possums, ringed seals, and llamas.

Surveys of *Giardia* in animal populations have often relied on detecting cysts in fecal samples, and these surveys may under-report the actual numbers of infected animals. In a survey of beaver and muskrat populations from the northeastern United States and Minnesota, Erlandsen et al.(1990b) 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.

IV. RISK ASSESSMENT

Giardia is frequently spread directly from person to person, especially among young children attending day-care centers, nurseries, institutions, or living in areas with poor sanitation and hygiene. *G. lamblia* infections in children attending day-care centers in developed countries are primarily asymptomatic with no adverse growth effects. Siblings are also an important risk factor for infection. For preschool children, the presence of a child older than 24 months in the household is important for risk of infection.

Although dogs and cats are often found infected, epidemiological studies have not found pets to be an importance source of infection. Several small foodborne outbreaks of giardiasis have been associated with the contamination of ice and foods by infected food service workers, but restaurant-associated transmission of *Giardia* does not appear to be a significant public health problem for children (Quick et al., 1992).

Waterborne transmission may be important. In the United States, *Giardia* is the most frequently identified etiologic agent causing waterborne outbreaks in public water systems. The relative importance of waterborne transmission among other risk factors for giardiasis, however, will vary among populations depending on general sanitation practices. For example, providing piped, high quality drinking water to some populations in developing countries may not significantly reduce the incidence of giardiasis even if contaminated drinking water is an important route of transmission (Esrey 1989). Other exposures from poor personal hygiene and inadequate sanitation may overwhelm the beneficial effect of clean drinking water.

Giardia is an important cause of both endemic and epidemic waterborne illness in the United States. Both visitors and residents have been affected in outbreaks. Children have been among the cases reported in waterborne outbreaks, but limited information is available on age-specific attack rates in waterborne outbreaks. In Berlin, New Hampshire, 38% and 60% of children under 10 years of age and children 10-19 years, respectively, were found infected during a waterborne outbreak (Lopez et al., 1980).

Other transmission routes and risk factors include secondary transmission among family members with young children, travel to endemic areas, and homosexual activities. Giardiasis can be transmitted by some sexual activities, particularly among male homosexuals who practice oral-anal sex (Farthing, 1996). 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). Giardiasis is common in populations living in poverty, with poor sanitation, and a high level of fecal contamination in the environment.

Hazard Characterization and Estimated Risk, United States

Prevalence/Incidence. Limited information from studies in Washington and Arizona suggest that over 1 million young children may be infected in the United States. Most will be asymptomatic.

Hospitalizations. An estimated 4,600 persons are hospitalized in the United States each year with giardiasis, and as many as 900 hospitalized cases may be children under 5 years of age. Limited information from studies in Michigan suggests that 170 of these hospitalized young children may be suffering from growth impairment.

Mortality. Mortality associated with *Giardia* infection is rare (Bennett et al., 1987). In the United States in 1982, giardiasis was listed as the underlying cause of death for four deaths.

Day-care transmission. In 1994, the Census Bureau estimated that 2,218,000 children attended day-care facilities in the United States. Epidemiological studies in various areas of the United States have found that 7% to 54% of children attending day-care centers are infected with *Giardia.* This suggests that 155,000 to 1,198,000 children attending day-care centers in the United States may be infected.

Secondary transmission. Infected infants and children, both symptomatic and asymptomatic, may infect other children and adults, e.g. family members or other care-givers. Secondary transmission of *Giardia* from children attending day-care centers is estimated to occur for 5% to 20% of household contacts and 9% to 35% of staff, and an additional 15,000 to 480,000 *Giardia* infections may occur in adults from contact with children in day care settings. Secondary transmission from children who are infected from waterborne exposures may also occur.

Waterborne transmission. *Giardia* has been the most commonly identified pathogen in waterborne outbreaks reported in the United States since 1971. During 1965 to 1996, 133 waterborne outbreaks and over 28,000 cases of giardiasis were reported in 27 states; 108 outbreaks were associated with the consumption of contaminated drinking water from public water systems, 10 outbreaks were associated with individual and nonpotable water sources, and 15 outbreaks were associated with recreational water. No deaths were associated with waterborne outbreaks. Because not all outbreaks are recognized or reported, these statistics should be considered an underestimate of the occurrence of outbreaks and incidence of waterborne giardiasis. Endemic or non-outbreak related waterborne giardiasis may also occur.

Eighty-four (74%) of the reported outbreaks were caused by inadequate treatment of surface water; 52 outbreaks occurred in surface water systems that were chlorinated but not filtered. Most of the outbreaks in unfiltered surface water systems occurred before EPA's Surface Water Treatment Rule (SWTR) was promulgated. Nineteen (17%) outbreaks occurred in filtered surface water systems. Fifteen percent 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.

Since 1971, 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.*

Epidemiological studies of endemic giardiasis have also reported waterborne risks. Persons who use shallow well or surface water sources for their household supply had twice the risk of giardiasis compared to persons who used either drilled wells or municipal sources. Persons using unfiltered municipal surface water systems had two to three times the risk of giardiasis compared to persons who used filtered municipal surface water systems. An estimated 155 million people of all ages (45 million are under 20 years of age) in the United States continue to use unfiltered surface water from municipal water systems. Those persons who use unfiltered surface water systems that do not meet provisions of the SWTR are considered at increased risk of waterborne giardiasis.

Outbreak investigations also show that *Giardia* is also frequently transmitted by ingestion of shallow well water, contaminated water while attending picnics, camping, and hiking, and water during swimming and

other water recreational activities. Poorly maintained wading and swimming pools and heavily used swimming areas at lakes and ponds pose an increased risk, especially if they are used by diaper-age toddlers or other persons prone to fecal accidents. Accidental ingestion during water play may be an important waterborne risk for young children. It is not known how many persons routinely use shallow well water or may be exposed to potentially contaminated swimming pools, wading pool, lakes, and streams.

Bennett et al. (1987) estimated that 120,000 cases of waterborne giardiasis may occur each year in the United States. In preparing this estimate, Bennett et al. (1987) queried personnel from the Centers for Disease Control and Prevention regarding the number of cases of reported and unreported giardiasis that may occur each year and presumed that 60% of all giardiasis is waterborne. The estimated number of waterborne cases is based on professional judgement and is likely to inflate the actual risk. Among the estimated 120,000 cases of waterborne giardiasis, some 34,500 cases are expected to occur each year in children. If 60% of hospitalized giardiasis cases are waterborne, 540 children under 5 years of age hospitalizated with giardiasis each year may be due to waterborne transmission.

An estimate that considers limited epidemiological data on the incidence of laboratory-confirmed cases during active surveillance of giardiasis from 1983 to 1986 in Vermont (Birkhead and Vogt, 1989) is similar to that of Bennett et al. (1987) if it is presumed that 60% of giardiasis is waterborne. The average annual incidence rate for giardiasis in all ages was 45.9 cases per 100,000 persons per year, higher than reported in other states (Birkhead and Vogt, 1989). If this incidence is applicable to the U.S. population, approximately 124,380 cases would be expected to occur each year with 42,000 cases occurring in persons under 20 years of age. If 60% of these cases are waterborne as Bennett et al. suggest, then about 75,000 cases of waterborne giardiasis are expected to occur each year with 25,000 of these cases in children.

Another estimate that considers data from passive surveillance during 1972 to 1973 in Colorado (Wright et al., 1977) is much lower and may be partially due to the under reporting of giardiasis in that study. Using the rate of giardiasis reported by Wright et al. (11.59 and 3.05 cases per 100,000 persons of all ages and persons under the age of 15 years, respectively), 31,500 cases of waterborne giardiasis would be expected each year with 1400 cases expected in children.

Birkhead and Vogt (1989) also estimated the incidence rates among persons whose residential water supply was either filtered or unfiltered municipal surface water. If the rate difference can be applied to the U.S. population, an estimated 21,000 cases of waterborne giardiasis are expected among persons that use unfiltered municipal surface water.

Rose et al. (1991b) used an exponential model to compute risks of *Giardia* infection from estimated exposures to *Giardia* in drinking water in the United States. Drinking water exposures were obtained from survey data of the occurrence of *Giardia* in polluted and pristine water sources and considered the average removal and inactivation of cysts with various types of water treatment. Annual risks of *Giardia* infection from drinking water, including asymptomatic infections, were found to average approximately 20×10^{-4} (20 infections per 10,000 people annually or 540,000 infections annually) and may be as high as 250×10^{-4} (250 infections per 10,000 people annually or 6,780,000 infections annually). These annual risk estimates are presented as point estimates without confidence limits, do not account for *Giardia* speciation and viability or analytical sensitivity and specificity, and have other limitations. If the dose-response curves for children and adults are similar and the quantitative risk assessment of Rose et al. (1991b) can be applied to children, 155,500 to 1,944,000 waterborne *Giardia* infections are expected annually among persons under 20 years of age. For

children under the age of five, 38,000 to 474,000 cases of waterbome giardiasis may occur. These estimates of infection are about 5 to 50 times greater than the Bennett et al. (1987) estimates of waterborne illness due to *Giardia*.

Teunis et al. (1997) recently completed a comprehensive risk assessment using Monte Carlo analysis, distributions rather than single estimates for levels of oocysts and cysts, analytical method recovery effectiveness, viability of the recovered cysts, removal of protozoa during water treatment based on *Clostridium* spores, and a daily consumption of 0.15 L/day tap water. The data used to develop the parameters were specific to the Netherlands with exception of the viability and the dose-response models. The cumulative estimate for an annual risk of waterborne infection in the Netherlands ranged from 10^{-5} to 10^{-4} for *Giardia*. The Rose et al. (1991b) estimate for the United States is 200 to 2500 times greater. The higher risks may be due to higher drinking water exposures in the United States and other limitations of the model.

V. OTHER CRITERIA, GUIDANCE AND STANDARDS

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 $\log_{10}$ (99.9%) for *Giardia* and 4 $\log_{10}$ (99.99%) for viruses through filtration and/or disinfection. 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.

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, 1994; U.S. EPA, 1996). 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 are: 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.

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 for environmental protozoa analyses. Until that time, adherence to specific methodological protocols, or performance of recommended quality assurance/quality control procedures, was strictly voluntary.

VI. ANALYTICAL METHODS

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.

Detection and identification in environmental samples. Classical cultural techniques, such as are used for bacteria and many of the enteric viruses, are not applicable to detecting, identifying and enumerating *Giardia* in environmental samples, and the probability that one could not be developed led to the development of microscopic examination methods. Large-volume water sample collection methods were developed using filtration through microporous cartridge media. Initially, flotation clarification techniques used zinc sulfate solutions; subsequently, other compounds including sucrose, Percoll, and Percoll-sucrose were evaluated and incorporated into the method. Voluntary efforts resulted in consensus methods to identify *Giardia* cysts; suspect organisms should possess the proper size and shape and at least two internal characteristics (nuclei, axostylar rods, median bodies).

All steps involved in the methodology including sample collection, elution, flotation clarification, and microscopic assay affect the recovery and detection of cysts. The sample collection and elution steps 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, significantly affect recovery. Higher turbidity in water being sampled was found to improve the retention of cysts on the sampling filter, but presented difficulties in the flotation purification and microscopic assay steps. The nature of the turbidity can also present problems. For example, algae can make clarification and detection more difficult.

The development of fluorescent antibodies for *Giardia* revolutionized the detection step which had previously been dependent upon examining concentrates with none-selective iodine staining. The fluorescent antibody assay, while improving detection of cysts, necessitated a new definition for identifying cysts. Presumptive cysts, defined by size, shape and apple green fluorescence under specified conditions of reagent and microscope configuration included all objects that might be *Giardia* cysts. The confirmed designation was applied to presumptive cysts with defined internal characteristics. This terminology, which created confusion for those not familiar with the methodology, was replaced by total counts of cysts and counts of cysts with internal structures in the *Standard Methods* and ICR methods.

Another limitation of fluorescent antibody identification is that the application and interpretation of results 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 the potential to specifically detect those species that may be important in human infection, and they have demonstrated improved sensitivity. However, problems have been encountered with reproducibility of the assays and with inhibition of the PCR reaction in environmental samples.

Increased awareness of method limitations has spurred development of alternative methods and procedures. In the area of sample collection, sampling and processing a 10 L volume instead of a portion of a 100 L or more is being investigated. Smaller sample volumes may result in fewer particulates to cause interferences in the detection assay and make 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 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.

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 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 which might require modifications depending upon local water quality conditions, equipment availability and analyst experience.

Viability. Determining the viability or infectivity potential of cysts detected with non-cultural methods has been difficult. A detected cyst may be either viable or non-viable. 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 determining the effectiveness of a treatment process to physically remove cysts, they are very important in assessing disinfectant efficacies or 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). Cysts stained with this PI 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.

For *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.

Dye staining, morphological criteria, and in vitro excystation may be adequate indicators of viability for some applications but can be conservative in estimating the potential for infection. Animal infectivity has commonly been used in experiments to determine disinfectant efficacy. However, animal infectivity may not be useful to evaluate the health significance of environmental isolates because some *Giardia* strains do not infect gerbils and the levels of detected cysts in a sample may be below the infectious dose for the animals.

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 relationship of viability and infectivity remains to be resolved.

Detection in biological samples. For diagnosis of giardiasis in either humans or animals, stools continue to remain the specimen of choice. Fresh stools can be used to prepare wet mounts that are examined

by conventional light microscopy for the presence of cysts or trophozoites. 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, 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. These kits have a high degree of sensitivity and specificity for a single stool sample. 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.

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.

Serodiagnosis is 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 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.

VII. TREATMENT TECHNOLOGIES

Because of the low infectious dose for *Giardia*, the wide-spread prevalence of infection in humans and a variety of animals, and the resistance of *Giardia* cysts to environmental conditions and water disinfectants, it is important to consider multiple barriers of protection: watershed management, filtration, disinfection, and protection of the integrity of the distribution system. Watershed management practices can reduce levels in surface waters, and well-head and aquifer protection programs can help prevent the entry of sewage and surface water in ground waters, but adequate water treatment is also required.

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 most disinfectants. For surface water sources and groundwater sources under the influence of surface water, both disinfection and filtration are recommended to effectively protect against the waterborne transmission of *Giardia*. Filtration can make disinfection more effective by reducing cyst levels, disinfectant demand, and particles that may interfere with disinfection effectiveness. A combination of water filtration and disinfection operated under optimum conditions can protect against waterborne transmission of giardiasis.

Filtration exceptions may be granted where water systems meet criteria specified by the SWTR; however, disinfection and disinfection by-products regulations may limit the concentration of chemical disinfectant that can be applied. 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.

Physical Removal

The majority of waterborne giardiasis outbreaks have occurred in unfiltered water systems, emphasizing the need for water filtration to reduce waterborne risks. Since outbreaks have also occurred in filtered water systems, good design, optimization of the filtration process(es), and frequent monitoring of treatment effectiveness are required to ensure effective removal of cysts.

The filtration technologies most frequently used to remove microbial contaminants and particles that cause turbidity are: conventional filtration, direct filtration, slow-sand filtration, diatomaceous earth (DE) filtration, and membrane filtration. Conventional and direct filtration, when operated under appropriate coagulation conditions, can remove 3 to 4 $\log_{10}$ (99.9% to 99.99%) of *Giardia* cysts. The highest removal rates were found in pilot plant studies and water utilities that optimized coagulation and achieved very low finished water turbidities (0.1- 0.3 nephelometric turbidity units or NTU). Cyst removal was poor in filtration plants where coagulation was not optimized even though the turbidity of filtered water was low. In some source waters, sedimentation may be needed to effectively remove cysts. 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.

Cyst removals by slow-sand and diatomaceous earth filtration are similar to or better than conventional and direct filtration, and operational and other factors are also important in maintaining high removals of cysts. 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. Membrane systems may require pretreatment to remove material that can clog or foul the membranes. 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.

Conventional and direct filtration. Physical-chemical interactions govern the attachment of suspended particle masses and *Giardia* cysts to the surface of sand and other filter media. Filtration by rapid granular filter media is not 100% effective, and cysts that are removed may become dislodged. An increase in turbidity or particles in the filter effluent may indicate the ineffective removal of cysts or the release of previously removed cysts. Each filter should be monitored to detect changes in water quality and assess its effectiveness.

Ongerth (1990) found that major deficiencies in the operation of three small water plants with either conventional filtration, direct filtration, or DE filtration caused poor removal of *Giardia* cysts. Bellamy et al. (1993) reviewed filtration 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). To maintain high filtration rates and removal effectiveness, granular 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 (e.g., filter to waste) before it is placed back online.

Al-Ani et al. (1986) demonstrated the importance of coagulation and optimum dosage of coagulant chemicals 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. When no chemical coagulation was used, removal of cysts was very poor, ranging from 0 to 50%; poor removals were also found when ineffective coagulants or improper dosages were used.

In pilot-scale studies, Logsdon et al. (1985) found that granular activated carbon, sand, coarse anthracite, and dual-media could remove 3 $\log_{10}$ of *G. muris* cysts. Decreased removals were associated with increased turbidity indicating filter breakthrough and also ocurred during the initial phase of the filter run emphasizing the need to provide for filter ripening. 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 slightly higher removals.

Catania 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 logs_{10} higher during treatment that 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 ripening.

Nieminski and Ongerth (1995) evaluated the removal of *G. lamblia* cysts in pilot- and full-scale water treatment plants. In the pilot-scale studies where the source water typically had turbidities of 4 NTU, 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, cyst removals averaged 3.3 $\log_{10}$ for conventional filtration and 3.9 $\log_{10}$ for direct filtration.

In a very low turbidity source water (0.33 to 0.58 NTU), Ongerth and Pecoraro (1995) found that 3.1 to 3.6-$\log_{10}$ of *Giardia* cysts were removed with optimal coagulation. When coagulation was suboptimal, cyst removal was only 1.3 $\log_{10}$ even though the filtered water turbidity was less than 0.5 NTU.

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.

LeChevallier et al. (1991a, b) reported that most of the 66 surface water filtration plants surveyed achieved between 2 and 2.5 $\log_{10}$ removals for *Giardia*. For treatment plants with *Giardia*-positive samples, an average of 2.14 $\log_{10}$ removal was found. 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 and Norton (1992) reported >2.3, >2.8, and >2.2 $\log_{10}$ *Giardia* removal at each of three locations where turbidities were high (1.8-120 NTU), moderate (3.5-75 NTU), and low (0.4-25 NTU) and the geometric mean number of cysts detected in raw water was 2.9, 5.8, and 9.1 cysts per L, respectively.

In two conventional water filtration plants with raw water turbidities of <0.1 to 60 NTU and <0.1 to 101 NTU, Kelley et al. (1995) observed a 1.5 $\log_{10}$ mean removal of *Giardia* cysts. The low removal was likely 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.

At three Montreal area conventional water filtration plants with geometric mean levels of 7, 336, and 1376 cysts/ 100 L in the raw water, Payment and Franco (1993) found >5 $\log_{10}$ removals of *Giardia* cysts. Sedimentation at the plants was shown to remove 2.7 to 2.9 $\log_{10}$ of *Giardia* cysts. States et al. (1995, 1997) reported that the Pittsburgh Water Treatment Plant removed 1.5 to 1.7 $\log_{10}$. *Giardia* cysts from the Allegheny River source where raw water samples contained a geometric mean of 58.6 cysts/100 L.

Slow sand and DE filtration. Sand in slow-sand filters has a much smaller effective size than that used in conventional or direct filtration and removal is accomplished by physical-chemical and biological mechanisms within the top layers of sand (Weber-Shirk and Dick, 1997). Generally, no pre-treatment is used with slow-sand filters, but some slow sand filters may be preceded by coagulation, sedimentation, or roughing filters which remove large size particles that may clog the filter.

Pilot-scale studies showed that removals of human-source *Giardia* cysts were uniformly high in slow sand filters at hydraulic loading rates of 0.04 to 0.4 m/h; removals averaged > 3 to 4 $\log_{10}$ (Bellamy et al., 1985). Removal is less efficient for slow-sand filters at near freezing temperatures (Fogel et al. 1993; Schuler et al., 1991).

DE filtration can also be used for 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. 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).

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 to 3 $\log_{10}$ removals could be achieved in the slow sand filter during the winter months, and a decreased removal efficiency occurred in the DE filter 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 reliance of effective removal by DE depends solely on proper operation. Studies with human-source *Giardia* cysts confirmed that DE filtration could remove >2 $\log_{10}$ of cysts (Jakubowski, 1990).

Membrane and Other Filters. Pressure-driven membrane filtration processes used for municipal water treatment are categorized by the effective size of the membranes (i.e., 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.

Jacangelo et al. (1991; 1995) found that hollow and spiral wound fiber microfiltration membranes and hollow fiber and tubular ceramic ultrafiltration membranes can achieve from 4.6 to >5.2 $\log_{10}$ removals of *G.*

muris 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.

Disinfection

Disinfectants can 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. *Giardia* 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 (MGOD), chlorine dioxide, iodine, free chlorine, and chloramines (Table 1).

Table 1. 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

Jakubowski (1990), Hoff (1986) and Jarroll (1988) reviewed the effectiveness of disinfectants to inactivate *Giardia* cysts. The effectiveness of disinfectants can be evaluated by *Ct* which 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. *Ct* values are available for various water temperatures and pH values in the SWTR Guidance Manual (U.S. EPA, 1989).

Chlorine. Studies by Jarroll et al. (1981) remind that chlorine does not always result in 100% inactivation of *Giardia* cysts and that the inactivation of cysts by chlorine is 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. At water temperatures of 5° C, exposures to 1 mg/L chlorine for 10 minutes contact time at pH 8 inactivated less than 45% of cysts. 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; at pH 7 and 8, less than 0.8% of cysts remained viable after 30 minutes. At 5° C, exposures to 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 8 mg/L killed 100% of cysts at pH 6 and 7 after contact for 10 minutes but required 30 minutes exposure at pH 8.

In general, the effectiveness of chlorination increases 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) noted erratic results in experiments with chlorine concentrations above 2.5 mg/L suggesting that *Ct* values calculated with high chlorine concentrations may not be reliable.

Rubin et al. (1989) evaluated the inactivation of human-source *Giardia* cysts by free chlorine using Mongolian gerbils; *Ct* values were generally found to be higher than previously reported. At 15° C the *Ct* values ranged from 5 to 62 at pH 9 and 139 to 182 at pH 5.

Chloramines. Chloramines are less effective than chlorine; *Ct* values for *G. muris* cyst inactivation by preformed monochloramine were substantially higher than those for chlorine at pH 7 and 5° C. Jarroll (1988) found *G. muris* cysts to be more resistant to chloramines at lower pH values; preformed chloramines were less effective than chloramines that are not preformed.

Chlorine dioxide. Using in vitro excystation as an indicator of viability, Finch et al. (1995) found chlorine dioxide inactivation of *G. muris* cysts was much more effective than free chlorine at 25° C and at pH 9. In contrast to findings with chlorine, chlorine dioxide effectiveness increases at higher pH values. At 25° C, the *Ct* value for chlorine dioxide ranged from 4.9-6.9 at pH 5 and a *Ct* of 1.7-3.0 at pH 9. 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).

Ozone. Ozone is more effective than chlorine for inactivation of either human-source *Giardia* or *G. muris* cysts and is less affected by water temperatures (Table 1). 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 at contact times of 2 and 5 minutes when 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).

Labatiuk et al. (1992) 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.

Mixed Oxidants. Witt and Reiff (1997) found that *Ct* values for an on-site disinfection process for small communities that uses electrolysis of a sodium chloride solution to produce a mixture of oxygen and chlorine species (MOGOD) were comparable to those for ozone and chlorine dioxide (Table 1).

Ultraviolet irradiation. Rice and Hoff (1981) 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*. Karanis et al. (1992) noted that UV disinfection is not reliable because commercial units 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.

Personal water purification. Jarroll (1988) found that six personal water purification methods employing iodine or chlorine disinfection were effective against *G. lamblia* cysts when prescribed procedures were employed for cloudy or clear water at water temperatures of 20° C. 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. (1989) 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., 1989).

VIII. ISSUES OF CONCERN

Risk assessment. 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 (Rose et al., 1991b). A model developed in the Netherlands addresses several of these limitations (Tennis et al., 1997), and a population-based model that considers incubation period, immunity, and secondary transmission has also been developed (Eisenberg et al., 1996). The EPA recommends a public health goal of no more than one *Giardia* infection per 10,000 persons from drinking water exposures (U.S. EPA, 1989). By requiring 99.9% reduction of *Giardia* cysts through filtration and/or disinfection of potentially contaminated water sources, the EPA presumed that this goal could be accomplished. However, the Rose et al. (1991b) risk assessment estimated that current risks in the United States are much greater than this recommended public health goal. If this risk assessment is correct, water utilities may be required to provide more than the minimum treatment for *Giardia* specified by the SWTR in order to meet the EPA recommended public health goal.

As more information becomes available from the ICR about the occurrence of *Giardia*, waterborne infection risks should again be estimated with each of the risk assessment models, and a sensitivity analysis should be conducted to identify the parameters that may have the greatest effect on risk estimates. Additional epidemiological studies are also needed to provide better quantitative information about the endemic waterborne risks of *Giardia* infection. Risks from well designed

epidemiological studies can be compared with estimates of risk from mathematical models and used to evaluate the whether EPA's recommended public health goal is being met.

Water treatment. *Ct* values published in the SWTR Guidance Manual (U.S. EPA, 1989) were based on results of laboratory studies in demand-free water and simplifying assumptions, such as the Chick-Watson relationship for microbial inactivation. Haas et al. (1996) cautioned that the type of source water may significantly affect predictions for microbial inactivation. Inactivation data obtained in laboratory studies using buffered demand-free water may be inadequate predictors of inactivation in actual waters to be disinfected, and the use of pH in adjusting *Ct* values may not be sufficient to characterize the effect of water quality on disinfection performance.

Under earlier designs and operating conditions, ultraviolet irradiation was not effective; however, recently developed systems may offer the potential for inactivating *Giardia* cysts (Budd et al., 1999; Clancey et al., 1999). Advanced systems provide the capacity for low-wavelength, high energy UV light and greater exposures to ultraviolet irradiation. Reports of their treatment efficacies may soon be available.

In response to the recent report that an isolate of *Flavobacterium* is capable of killing *Giardia* cysts (Rodgers et al., 1998), research is encouraged on the development and application of biological control agents for wastewater and drinking water treatment. This or other bacteria may be useful as a biological control agent for *Giardia* in source waters.

Occurrence and analytical methods. With the recent increased emphasis on the effectiveness of water disinfection and filtration to inactivate and remove *Cryptosporidium* oocysts, less attention has been paid to *Giardia*. The assumption is often 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*. 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 cysts and oocysts may not be the same.

Although the ICR database will soon be available on the occurrence and distribution of *Giardia* in a variety of waters, the sources of contamination on specific watersheds and the factors affecting fate and transport of cysts are not well characterized. Studies should be conducted to determine water quality changes associated with the various watershed management practices to reduce non-point sources of contamination. For example, giardiasis is more common in immature animals, and reduction and relocation of suspected animal sources, such as beaver, may have unintended consequences. 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.

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.

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.

Health effects. Anti-giardial drugs are important in the management of individual cases of giardiasis, but they may not prevent frequent reinfection of children who attend day-care centers or live in communities where *Giardia* exposures are frequent. It is questionable whether a vaccine can be developed for human use, and even if one can be developed, its use will likely be limited.

IX. RESEARCH NEEDS

Zoonotic routes. Additional information is needed to answer questions about *Giardia* species and their zoonotic routes of transmission. The characterization of *Giardia* by molecular approaches, such as zymodeme or karyotype identification, can be useful in this regard. Carefully controlled animal feeding studies and more comprehensive epidemiological investigations of outbreaks and endemic risks can provide additional information about important animal reservoirs of infection.

Environmental occurrence. 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.

More research should be conducted 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.

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

Research is needed on methods to quantitatively detect occurrence and survival of *Giardia* on various fruits, vegetables, and foods that are usually consumed without cooking. With the increased globalization of our food supply, additional surveillance of these foods is desirable.

Recent studies have demonstrated that significant fractions of *Cryptosporidium* oocysts can survive temperatures just below freezing for relatively long time periods. Similar data should be developed for *Giardia*.

Information on the occurrence and survival of *Giardia* cysts in estuarine environments is limited and additional research should be conducted in this area.

Human health effects. 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. More information is needed about waterborne exposures to *Giardia* cysts. This includes studies of the occurrence, transport, and fate of cysts in drinking water sources, storm waters, reservoirs, animal waste ponds and lagoons, and septic tank effluents.

Additional research is needed to better describe the role of protective immunity in symptomatic illness and how long immunity might last. The immunologic determinants for clearance of acute infection and development of protective immunity are not well defined. 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.

Additional research is needed on the suitability of using saliva for detection of anti-*Giardia* antibodies in patients with giardiasis. Saliva tests can be more easily applied to studies of children than serological tests.

Most prevalence studies have been conducted in developing countries, and additional data are needed to better assess the current prevalence of *Giardia* infection, especially among children in the United States.

Breast milk may protect some infants from *Giardia* infection because of protective immunity of secretory antibodies in breast milk or because of breast milk enzymes that have been found *in vitro* to release substances which kill *Giardia* trophozoites. The use of breast milk also results in fewer exposure opportunities for the infant. Further study is required to better define the significance of these factors in protecting breast-fed infants from infection.

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Additional research is needed to help clarify the association between giardiasis and growth impairment so that children at greatest risk of growth retardation can be identified. The role of transient or permanent immune deficiencies in increasing the risk of growth retardation from *Giardia* infection should also be investigated. Other observed associations with *Giardia* infection that require additional study include allergic reactions, cystic fibrosis, synovitis, pancreatic or hepatic disease, and ocular changes.

Although current drugs have been found effective in the treatment of giardiasis, resistance has been observed for certain strains or genotypes. An alternative is to treat giardiasis with drugs aimed at the metabolism of *Giardia*. Research on its unique metabolism might suggest a way to interfere with its life cycle.

Water treatment. 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.

Research is needed on the inactivation of *Giardia* cysts by the newer UV processes and other electrotechnologies. 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.

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X. REFERENCES

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