APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1989, p. 1223–1229
0099-2240/89/051223-07$02.00/0

Vol. 55, No. 5

Viability of *Giardia* Cysts Suspended in Lake, River, and Tap Water

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Received 3 October 1988/Accepted 10 February 1989

Numerous waterborne outbreaks of giardiasis have occurred since 1965, yet little or no information has been reported on the viability of *Giardia* cysts in different aquatic environments. We have studied the viability of *Giardia muris* cysts suspended in lake, river, and tap water, while also monitoring water temperature, dissolved oxygen, pH, and other water quality parameters. Fecal pellets containing *G. muris* cysts were placed in glass vials covered with filter paper and exposed to (i) lake water at 15 ft (ca. 4.6 m) and 30 ft (ca. 9.2 m), (ii) river water, (iii) tap water, and (iv) distilled water stored under laboratory conditions. At 3, 7, 14, 28, 56, and 84 days, two vials from each environment were removed, and cyst viability was determined by (i) fluorogenic dye exclusion, (ii) production of giardiasis in an animal, and (iii) cyst morphology by Nomarski microscopy. In the fall, the cysts suspended at 30 ft in lake water remained viable for up to 56 days whereas cysts stored at 15 ft were nonviable after day 28. The *G. muris* cysts exposed to river water remained viable up to 28 days as determined by the production of giardiasis in mice. *G. muris* cysts suspended in tap water showed no signs of viability after 14 days, while cysts serving as controls (exposed to refrigerated distilled water) remained viable for up to 56 days. In the winter, *Giardia* cysts suspended in either lake or river water were viable for 56 to 84 days whereas cysts exposed to tap water were nonviable by day 14. Comparison of water quality parameters with the results of viability determination revealed that only decreased water temperature (<10°C) was consistent with prolonged survival of *G. muris* in different types of environmental water.

During the past 20 years there has been a sharp increase in the number of waterborne outbreaks produced by ingestion of the cysts of the intestinal protozoan *Giardia*. Many of these occurrences have been associated with the ingestion of untreated surface water by hikers and campers (10, 11). A number of cases have also been derived from water in mountain streams or wells serving as water sources for ski resorts (22, 25, 26). However, giardiasis outbreaks have not been limited only to people who ingest untreated surface water, since in many municipalities using only chlorine for disinfection, outbreaks of giardiasis have been reported after the consumption of the treated water (19).

The source of *Giardia* cysts found within surface water has caused great concern, and the evidence for contribution of various animals to cysts in the water has been recently evaluated (13). Davies and Hibler (12) suggested that animals having natural access to water, such as beavers, dogs, cats, cattle, and humans, might be reservoirs for *Giardia*, while others have implicated muskrats (13, 20), voles (20, 28), coyotes (12), and some birds (6, 20). Owing to a lack of convincing evidence, the role of each of these animals in contributing to waterborne outbreaks of giardiasis has remained controversial (13).

Waterborne outbreaks of giardiasis have been reported by Craun (11) to occur throughout the entire year, suggesting that the water may be contaminated on a frequent basis or that cysts may survive for long periods of time in water. Lippy (21) has calculated that one human stool, deposited in a medium-sized reservoir (i.e., 2.5×10^6 gal [1 gal = 3.785 liters]), could lead to a density of 20 to 25 cysts per gallon. On the other hand, it is possible for the water to be contaminated on an ongoing basis, since Hibler et al. (16) have reported that 40 to 45% of raw water samples examined were often contaminated with *Giardia* cysts during the months from fall to spring.

Very little information has been reported on the influence of temperature on the viability of *Giardia* cysts under either laboratory or environmental conditions. Bingham et al. (1) used both eosin exclusion and in vitro excystation to investigate the effect of temperature on the viability of *G. lamblia* cysts stored in distilled water (dH_2O) and showed that cysts stored at 37°C never survive longer than 4 days, whereas cysts stored at 8°C are viable for up to 77 days in dH_2O. Despite the widespread interest in the viability of *Giardia* cysts, particularly in light of the potential risk of disease that exists with surface water contaminated with *Giardia* cysts, no previous studies have been found describing the viability of *Giardia* cysts in water found outside of the laboratory.

In this study, the viability of *G. muris* cysts suspended in water was used as a model for the survival of *G. lamblia* cysts, since it has been reported by Rice et al. (30) and Jarroll (18) that the former were more resistant to disinfection agents in vitro and also because the introduction of *G. muris* cysts into water poses no risk of infection to humans. *Giardia muris* cysts were suspended in three different types of water found in the environment, including lake, river, and tap water. Using the exclusion of fluorogenic dye by cysts (33, 35) and the ability of *Giardia* cysts to produce infection in an animal as measures of viability, it was found that *G. muris* cysts remained viable for 1 to 3 months depending on the temperature and type of water sample.

MATERIALS AND METHODS

Fecal pellets were collected from infected 14- to 21-day-old CF-1 non-Swiss mice (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) shedding *G. muris* cysts. The formed fecal pellets were placed in glass vials (22 by 39 mm; volume, 8 ml; Wheaton Scientific, Millville, N.J.) and filled with dH_2O. The fecal mass in each vial contained approximately

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4×10^5 cysts, as determined by hemacytometer count. The openings of the vials were covered with pieces of filter paper (grade 615, Whatman; VWR Scientific, Inc., Chicago, Ill.) and were held in place by the plastic lids, which had their centers removed. The filter paper facilitated the diffusion (of water, aquatic life, chemicals, etc.) into and out of the vials. Three types of water at different environmental sites were tested for their effects on the viability of the *G. muris* cysts.

Environmental test sites. (i) Lake water. Groups of glass vials containing the fecal pellets were placed in a perforated polyvinyl chloride container and were separated from one another by circular pieces of porous mesh to permit exchange of water. During the fall sampling period (31 July 1987 to 25 September 1987), one container was suspended from a buoy in 15 ft of lake water while a second container was suspended, below the thermocline, in 30 ft of water. During the winter months (10 December 1987 to 2 March 1988), the sample containers were fastened to the base of a hollow aluminum pipe. The retrieval of samples through the ice was facilitated by heating the aluminum pipe and then withdrawing it and the polyvinyl chloride containers through the hole in the ice.

(ii) River water. In cooperation with the Minneapolis Water Works, a polyvinyl chloride container with sample vials similar to the one used in the lake was suspended in about 10 ft (ca. 3 m) of water at an inactive water intake in the Mississippi river. Retrieval of the samples during the winter was achieved in a similar manner to that used for the lake water samples.

(iii) Tap water. Glass sample vials containing fecal pellets were placed in a Nalgene rack and submerged in a stainless steel tank which was continuously perfused, using reverse flow, at a rate of approximately 2 liters/min. The temperature of the flowing tap water (cold water setting only) reflected seasonal variations in the city water supply, and no external temperature regulation was used.

(iv) Refrigerator. Sample vials submerged in dH_2O were placed in a standard laboratory refrigerator and maintained at 5 to 7°C.

Water parameter testing. A Van Dorn water sampling device was used to collect lake and river water samples from the depth at which the sample vials were suspended. The water parameters tested on all samples included temperature, pH, and dissolved oxygen (DO_2), which were determined at the same time the samples were retrieved. The temperature and DO_2 were measured with a combination temperature-DO_2 meter (model 9070; Jenway Ltd., Essex, England). The pH was measured by using a portable pH meter, (model 612; Markson Scientific, Phoenix, Ariz.). The other water quality parameters were performed by the Minneapolis water treatment laboratories and included determination of total hardness by the EDTA titrimetric method (3), measurement of nitrate by the nitrate-nitrite cadmium reduction method (23), nitrogen (ammonia) determination by the Nesslerization method (7), and determination of phosphorous by the single reagent method (24). Turbidity was measured by using a ratio-XR Nephelometer, (model 43900 Hach Co., Loveland, Colo.), and color determination was accomplished by a potassium chloroplatinate visual comparison method (8). The lake and river water sites were also assayed for the presence of *Giardia* cysts indigenous to these two natural environments by following the water collection procedure recommended by Environmental Associates, Ltd., Limestone, N.Y. Briefly, 150 to 200 gallons (ca. 568 to 757 liters) of water was filtered through a wound yarn filter (Micro-Wynd II filter; cartridge DPPPY; 1-μm nominal rating 69331-38; AMF Cuno, Meriden, Conn.). The filter was shipped to Environmental Associates, Ltd., and examined by microscopic techniques, including fluorescent-antibody methods, for the presence of *Giardia* cysts.

Sample handling. At 3, 7, 14, 28, and 56 days after the fall and winter trials were begun, and also at 84 days during the winter trial, two sample vials were removed from each environmental test site and placed on ice. Each sample was examined microscopically to determine the presence of *Giardia* cysts. Viability of *Giardia* cysts in each sample was determined by fluorogenic dye exclusion by the method of Schupp and Erlandsen (33) and also by determining animal infectivity by using the mouse model described by Roberts-Thomson et al. (31).

Fluorogenic dye exclusion. Viability of the *Giardia* cysts was determined by exclusion of the fluorogenic dye propidium iodide (PI) as previously described (33). Briefly, 1 ml of a fecal slurry obtained from each sample vial was incubated with 0.04 ml of 0.015 mM PI for 10 min at 0 to 4°C. A cover slip was placed over the sample, and the sample was examined by using an Olympus BH-2 microscope equipped with a 100-W high-pressure mercury epi-illumination unit. Viable cysts excluded PI, while dead cysts displayed nuclear and cytoplasmic staining when observed by using a 545-nm (green) exciter filter along with a 580-nm chromatic beam splitter and a 590-nm barrier filter. The percentage of viable cysts was calculated by counting 100 cysts per sample or, in samples with sparse numbers of cysts, the number of cysts that could be counted in 30 min, (i.e., never less than 15 to 20 cysts per sample). These numbers were used in the calculations. The samples containing the low numbers of cysts were generally the samples exposed to water for longer than 56 days.

Animal infectivity. At each time point and from each water test site, a 1.0-ml sample of the fecal slurry taken from each of two duplicate sample vials was combined and 0.1 ml was given intragastrically, via a plastic stomach tube, to each of five or six 14- to 21-day-old CF-1 non-Swiss mice. Six mice were left uninoculated, as controls. The groups of mice were kept in separate cages to prevent the possibility of cross-transmission between animals. At 4 and 5 days postinoculation, the feces of each mouse was checked microscopically to determine the presence of *Giardia* cysts. If cysts were not detected in the feces, then the mice were sacrificed on day 5 by cervical dislocation, and the small intestines were examined by light microscopy for the presence of motile trophozoites.

RESULTS

Giardia cysts were detectable in every fecal sample (suspended in water) from all environmental test sites and at all time points analyzed. However, it was observed that the numbers of cysts decreased over time, as determined by the difficulty of microscopic detection of the cysts. The number of *G. muris* cysts in each sample vial at the beginning of the winter trial averaged approximately $1.4 \times 10^5 \pm 4.1 \times 10^4$ cysts per vial. After 28 days, the number of cysts had declined to $7.1 \times 10^4 \pm 4.4 \times 10^4$, while at 56 days of the winter trial, the average number of cysts was $3.6 \times 10^4 \pm 1.8 \times 10^4$. Nonviable *G. muris* cysts, were easily detected by PI incorporation but often were more difficult to identify by bright-field microscopy partly because of the deterioration in the morphologic appearance of the cysts and also because of visual interference with adherent particulate matter. Be-

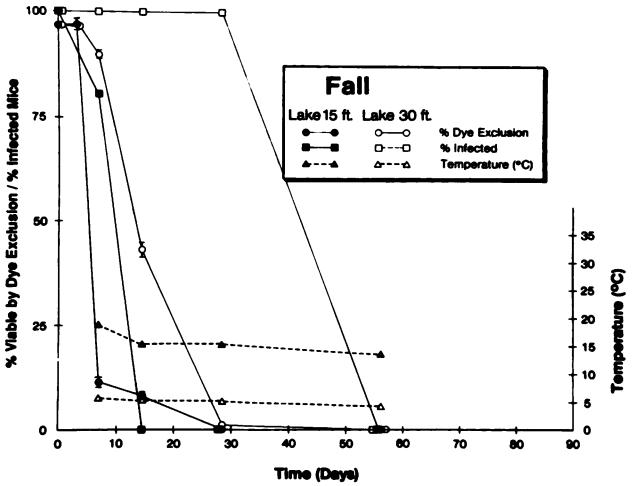

FIG. 1. The effect of lake water during the fall on the viability of *G. muris* cysts as determined by exclusion of the fluorogenic dye PI and the production of infection in mice.

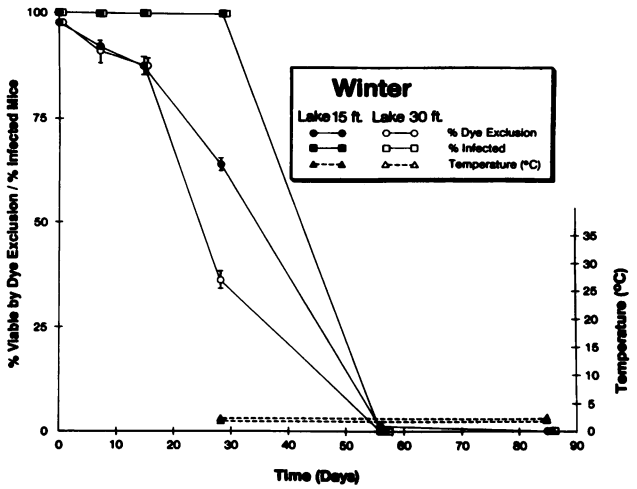

FIG. 2. The effect of lake water during the winter on the viability of *G. muris* cysts as determined by exclusion of the fluorogenic dye PI and the production of infection in mice.

cause no *Giardia* cysts were found to occur naturally within samples of lake or river water, all cysts observed in our test samples were interpreted as being derived from the suspended murine fecal pellets containing *G. muris* cysts.

The fecal pellets containing *Giardia* cysts that were stored in refrigerated dH_2O (5 to 7°C) served as controls. After 7 days of storage at this temperature, the viability of the *G. muris* cysts was measured by dye exclusion and found to be 90% in the fall trial and 83% in the winter trial. After 28 days, the viability of the cysts was measured again by dye exclusion and found to be 25% in the fall trial and 13% in the winter trial. By 56 days, the viability of the cysts as measured by PI had dropped to <1% in both the fall and winter samples. Using the mouse model of infectivity as a second measure of viability, it was demonstrated that at day 7 in both fall and winter trials 100% of the mice (n = 6) inoculated with a sample of fecal slurry from the refrigerated sample became infected. After 28 days, 17% of the mice (n = 6) given *G. muris* cysts stored in dH_2O in the fall trial were positive for *G. muris* infection, while 100% of the mice (n = 6) in the winter trial were infected. However, by day 56, none of six mice inoculated with the slurry from any of these samples were positive for giardiasis.

Viability of *G. muris* cysts in lake water. The relationship of water depth and water temperature with the viability of *G. muris* cysts over time is shown in Fig. 1 and 2. During the fall (Fig. 1), the temperature of the lake water at the sample depth of 15 ft ranged from 17 to 20°C, while the water temperature below the thermocline for samples at 30 ft of water fluctuated between 6 and 7°C. At day 7 of the fall trial, 12 and 91% of the *G. muris* cysts observed in the samples at 15 and 30 ft, respectively, were demonstrated to be viable by the PI dye exclusion method. At this same time point, 80% (n = 5) and 100% (n = 6) of the mice became infected when given a 0.1-ml inoculum of fecal slurry made from fecal samples suspended in lake water at 15 and 30 ft, respectively. On day 28 of the fall trial, all *G. muris* cysts observed in the sample suspended at 15 ft were shown to be nonviable by both PI dye exclusion and failure to produce giardiasis infection in mice. Of the *G. muris* cysts detected in the sample suspended in the lake at 30 ft, 1% were shown to be viable by PI dye exclusion, and the inocula from these samples produced infection in 100% of the mice (n = 6). By day 56 of the fall trial, *G. muris* cysts were detected morphologically within samples suspended at both 15 and 30 ft, but none of the cysts were shown to be viable by either dye exclusion or by the production of infection in mice.

During the winter trial (Fig. 2), the temperature of the lake water at 15 and 30 ft ranged from 2.9 to 3.3°C. At day 7 of the trial, the viability of the *G. muris* cysts from samples at either depth was measured by dye exclusion and found to be >90%, while 100% of the mice (n = 6) inoculated with a sample of fecal slurry from each of the water depths became infected. At day 28 of the winter trial, 63 and 36% of the *G. muris* cysts detected in the samples suspended at 15 and 30 ft within the lake, respectively, were demonstrated to be viable by the PI dye exclusion method and produced giardiasis in 100% of the inoculated mice (n = 6 for each depth). At day 56 of the winter trial, the sample obtained at 15 ft had viable *G. muris* cysts (<1%) as shown by dye exclusion. Neither sample, whether obtained at 15 ft or 30 ft, was able to produce infections when injected into mice. No viable cysts were detected either by dye exclusion or by animal infectivity in the samples suspended in lake water for 84 days.

Viability of *G. muris* cysts in river water. The effect of the water temperature on the viability of *G. muris* cysts exposed to river water is shown in Fig. 3 and 4. The temperature of the river water during the fall (Fig. 3) ranged between 19 and 27°C, while the water temperature during the winter (Fig. 4) remained between 0 and 2°C. The viability obtained after 7 days of exposure to river water during the fall was determined to be 1%. Of the mice (n = 5) given an inoculum of a fecal slurry containing cysts, 80% became infected with *G. muris*. At day 28 of the fall trial, none of the *Giardia* cysts detected in the samples exposed to the river water were shown to be viable by PI dye exclusion. However, 17% of the mice (n = 6) inoculated with a slurry prepared from this sample became infected. At some unknown time point after day 28, the entire sample container disappeared from its site; therefore, no sample could be analyzed at day 56. During the winter trial, the viability of *G. muris* cysts measured by dye exclusion was >84% at day 7 and decreased to 71% at day 28. It then dropped to 9.5% at day 56. At 7, 28, and 56 days, 100% of the mice (n = 6 at each time point) that had been inoculated with *G. muris* cysts from the water samples

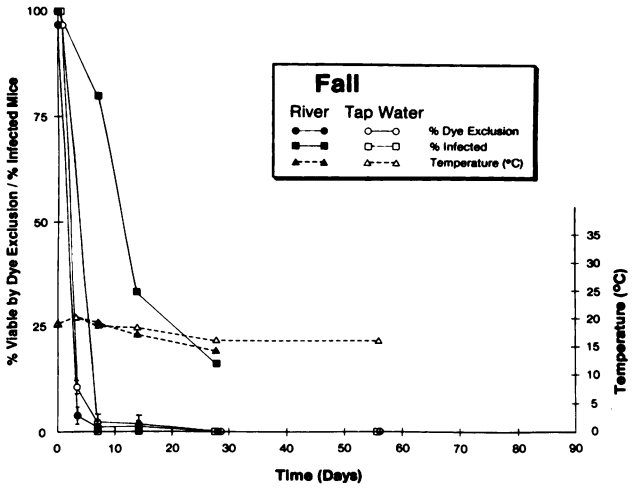

FIG. 3. The effects of river water and tap water during the fall on the viability of *G. muris* cysts as determined by exclusion of the fluorogenic dye PI and the production of infection in mice.

became infected with *Giardia*. However, on day 84 in the winter trial, the *Giardia* cysts recovered were nonviable, as judged by both dye exclusion and the lack of infection in mice inoculated with samples of the cyst-containing fecal slurry.

Viability of *G. muris* cysts in tap water. The temperature of the tap water used for suspending the *G. muris* cysts was relatively constant throughout both the fall and winter trails (20 to 28°C). The relationship between the water temperature and viability of the *Giardia* cysts is shown in Fig. 3 and 4. As judged by dye exclusion, the viability of the *G. muris* cysts exposed to tap water dropped to <2% at day 7 in both the fall and winter trials. No mice ever became infected when inoculated with cysts exposed to tap water during the fall, while only 17% of the mice (n = 6) became infected at day 7 in the winter trial. No viable cysts were detected after day 14 by either dye exclusion or animal infectivity.

The average values for the water quality parameters tested during both the fall and winter trials at all environmental sites are listed in Table 1. Of all the parameters tested, only the water temperature showed any probable relationship to the viability of *G. muris*. This relationship can be seen in Fig. 1 to 4. The prolonged temporal viability of the *G. muris* cysts seemed to be associated with an environmental water temperature of <10°C, while the cysts exposed to water temperatures of >10°C showed a decreased temporal viability when measured by both PI dye exclusion and mouse infectivity.

The average measurements of the water DO_2 content, turbidity, color, and hardness fluctuated widely. However, these differences in water parameter measurements appeared to be of very little value for use in predicting the viability of the *G. muris* cysts stored in water found in the environment. For example, in a sample of lake water at a depth of 30 ft, the average DO_2 was 1.2 mg/liter during the fall trial (28 days) and 10.2 mg/liter for the river sample in the winter trial (56 days), yet cyst viability as measured by mouse infectivity was 100% for both samples despite the 10-fold difference in DO_2. Similar results were seen when the extreme values of turbidity, color, and hardness were considered, indicating that these parameters had little affect on the viability of the cysts. The average values for ammonia, nitrate, and phosphorous at all times tested did not vary widely, regardless of the type of water, and therefore did not appear to correlate with the changes in cyst viability.

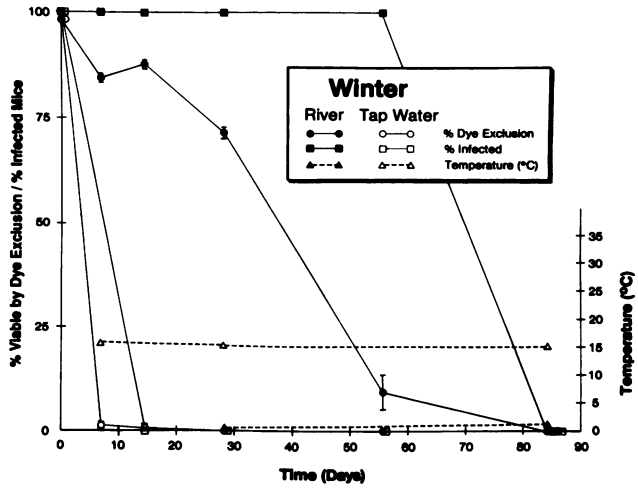

FIG. 4. The effect of river water and tap water during the winter on the viability of *G. muris* cysts as determined by exclusion of the fluorogenic dye PI and the production of infection in mice.

DISCUSSION

The results of this study demonstrate for the first time the effect on the viability of *G. muris* cysts of three different water sources during two different seasons of the year. Previous studies on the viability of *Giardia* cysts have included only experiments using cysts that have been stored under laboratory conditions. The viability of *Giardia* cysts exposed to lake water (two different depths), river water, and tap water along with cysts stored in dH_2O were measured by using dye exclusion with PI and the ability of cysts to produce infection in mice. It could be shown that the *G. muris* cysts exposed to water temperatures of <10°C (e.g., in lake water at 30 ft or stored in refrigerated dH_2O during the fall, as well as all the samples in the winter trial except tap water) retained viability for between 1 and 3 months. The viability of the cysts exposed to water temperatures of >10°C (e.g., in lake water at 15 ft and river water during the fall, along with tap water in both trial seasons) was reduced when compared to cysts exposed to water temperature of <10°C. It is important to note that of the nine water quality parameters tested, only the temperature of the water had any apparent correlation with the viability of the cysts.

Previous studies on the influence of temperature on the viability of *Giardia* cysts in water have been conducted under laboratory conditions by using the methods of eosin dye exclusion and excystation. Using the eosin exclusion method to determine cyst viability, Boeck (4) reported the thermal death point of *G. lamblia* cysts to be 64°C, while Cerva (9) found the death point to be 50°C. In another experiment Boeck (5) found that *G. intestinalis* cysts stored in dH_2O retain viability for 66 days as determined by eosin exclusion. By dye exclusion, *Giardia* cysts stored at 12 to 22°C were shown by Boeck (5) to be viable for up to 66 days, while Naik et al. (27) demonstrated that *G. lamblia* cysts in water at 4°C remain viable for up to 90 days. However, the reliability of using trypan blue or eosin dye exclusion methods for measuring the viability of *Giardia* cysts in vitro has been shown by Bingham et al. (1) to be relatively inaccurate. Using excystation to determine *G. muris* cyst viability,

TABLE 1. Seasonal comparison of water quality parameters of environmental water used to test *G. muris* cyst viability

Water sample	Temp (°C)[a]	pH[a]	DO_2 (mg/liter)[a]	Turbidity[b]	Color[b]	Hardness (mg of $CaCO_3$/liter)[b]	Ammonia (mg/liter)[b]	Nitrate (mg of N/liter)[b]	Phosphorus (mg of PO_4/liter)[b]
Lake									
15 ft									
Fall	19.2 ± 1.3	7.7 ± 0.2	3.0 ± 0.9	2.4 ± 0.4	28.5 ± 1.5	170.5 ± 1.5	0.3[c]	ND[d]	0.1
Winter	3.0 ± 0.1[b]	6.6 ± 0.2[b]	4.4 ± 0.4[b]	0.8 ± 0.1	20.5 ± 0.5	197.0 ± 3.0	0.3 ± 0.01	0.6 ± 0.1	0.1 ± 0.01
30 ft									
Fall	6.6 ± 0.4	7.3 ± 0.1	1.2 ± 0.3	6.6 ± 4.5	28.0 ± 0.0	188.5 ± 9.5	0.8[c]	ND	0.6[c]
Winter	3.2 ± 0.1[b]	6.6 ± 0.4[b]	4.2 ± 0.2[b]	1.0 ± 0.6	20.0 ± 0.0	197.5 ± 0.5	0.3 ± 0.1	0.6 ± 0.1	0.1 ± 0.01
River									
Fall	23.0 ± 3.1	8.4 ± 0.1	4.9 ± 0.5	9.0 ± 0.6	49.0 ± 6.0	172.5 ± 3.5	0.9[c]	ND	<0.1[c]
Winter	0.7 ± 0.4[b]	6.8 ± 0.1[b]	10.2 ± 0.1[b]	4.9 ± 2.9	18.0 ± 0.0	194.5 ± 2.5	0.5 ± 0.1	0.6 ± 0.1	0.1 ± 0.1
Tap water									
Fall	24.7 ± 2.0	8.2 ± 0.1	6.2 ± 0.7	0.7 ± 0.1	4.5 ± 1.5	73.5 ± 3.5	0.2[c]	ND	0.4[c]
Winter	20.8 ± 0.2[b]	8.2 ± 0.4[b]	9.2 ± 0.8[b]	0.2 ± 0.0	3.0 ± 1.0	88.5 ± 4.5	1.3 ± 0.2	0.6 ± 0.1	<0.1 ± 0.1
dH_2O									
Fall	7.1 ± 0.5	ND	ND	0.1 ± 0.1	2.0 ± 0.0	1.5 ± 1.5	0.1[c]	ND	<0.1[c]
Winter	5.4 ± 0.2[b]	5.0 ± 0.1[b]	6.0 ± 0.6[b]	0.2 ± 0.1	2.0 ± 0.0	17.5 ± 14.5	0.1 ± 0.1	<0.1 ± 0.1	<0.1 ± 0.01

[a] Values are the means of three or more determinations unless otherwise noted.
[b] Values are the means of two determinations unless otherwise noted.
[c] Values are the results of a single determination.
[d] ND, Not determined.

Schaefer et al. (32) found the thermal death point to be 54°C and also observed that cysts stored in dH_2O were viable for at least 40 days. Bingham et al. (1) used excystation as the criterion for *Giardia* cyst viability and found that *G. lamblia* cysts remain viable for up to 77 days when stored in dH_2O. At 8°C, they showed that *Giardia* cysts excystation rate varies from 10 to 55% between 7 and 37 days but drops to low levels (approximately 1%) after 37 days, with a similar low number of cysts being detected until 77 days (1). Using a gerbil model of giardiasis as the criterion for viability, Visvesvara et al. (36) recently reported that *G. lamblia* cysts "could be stored [under laboratory conditions] in the cold for up to 34 days with no loss of infectivity." Observations by Fantham and Porter (15) suggest that *G. lamblia* cysts isolated from human feces and stored for 74 days can be infective for kittens (n = 2); however, no information was provided as to what aqueous medium the cysts were stored in or the storage temperature. Our results, using *G. muris* cysts as a surrogate for *G. lamblia* cysts, have demonstrated that the survival of *Giardia* cysts in environmental watersheds was similar to those reported for laboratory environments. *G. muris* cysts suspended in environmental water remained viable for 2 to 3 months, and their survival was enhanced by exposure to low water temperature, despite the fact that the cysts were suspended in the fecal biomass within the sample vial.

The determination of the viability of *Giardia* cysts can be determined by using four different methods, including (i) the uptake or exclusion of fluorogenic dyes (33), (ii) the morphology of cysts by light microscopic (34), (iii) in vitro excystation (2, 17), and (iv) the production of giardiasis in an animal (17, 31). Frequently these methods for determining viability have been used interchangeably, particularly in determining the survival of cysts exposed to chlorine. This has led to disparate values for contact time with chlorine when viability measured by excystation and animal infection are compared (P. M. Wallis, P. D. Roach, J. M. Buchanan-Mapin, A. VanRoodselaar, B. Grey, F. Skinner, and J. S. Davies, manuscript in preparation). The general relationship of *Giardia* viability as determined by fluorogenic dyes, excystation, and infection has been schematically represented as overlapping areas in Fig. 5. It should be noted that all cysts capable of producing an animal infection would have displayed viability as measured by either the use of fluorogenic dyes or excystation. However, not all cysts that exclude the fluorogenic dye PI or incorporate the dye fluorescein diacetate may be able to undergo excystation.

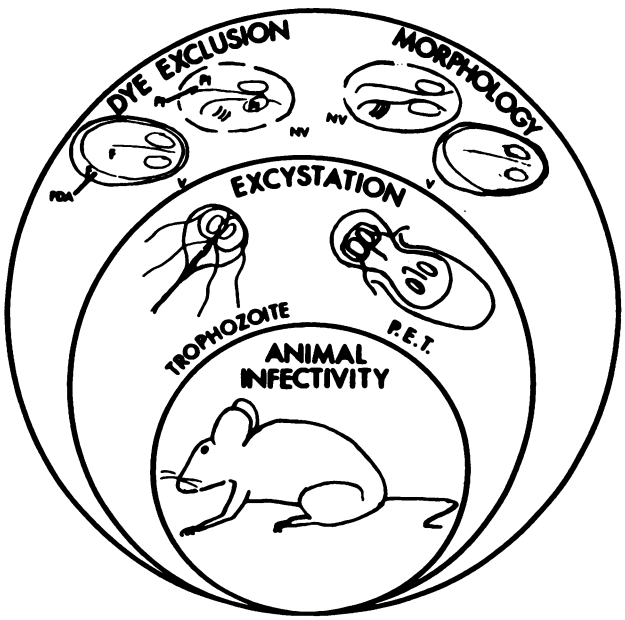

FIG. 5. Schematic diagram illustrating the relationship between *Giardia* cyst viability as determined by use of fluorogenic dye, excystation, and production of animal infection. FDA, Fluorescein diacetate; P.E.T., partially emerged trophozoite; V, viable; NV, not viable.

Similarly, the estimation of viability as determined by excystation includes by definition (2, 32) not only the tabulation of free-swimming trophozoites but also what are referred to as partially emerged trophozoites. Many of the partially emerged forms appear never to complete the excystation process; therefore, counting partially emerged forms as viable cysts may result in an overestimation of the viability of a population of *Giardia* cysts when excystation values are compared with infectivity (P. M. Wallis, personal communication). In our study on the effect of environmental water on the viability of *Giardia* cysts as a function of time, we chose two methods for measuring viability. One method was the use of the exclusion of the fluorogenic dye PI, since it provides a general determination of living cells with intact cell membranes (33, 35). As a second measure of cyst viability, the ability of *Giardia* cysts to produce infection in an animal was used, since it would include not only dye exclusion and excystation, but more important, it would measure the ability of cysts to survive exposure to water and produce giardiasis in animals. A comparison of *Giardia* cyst viability using these two methods (Fig. 1 to 4) shows a relatively good correlation between the methods. However, the viability of cysts exposed to different types of water as determined by infectivity in the mouse model cannot be compared directly to the viability of cysts determined by dye exclusion, since as few as 5 to 10 cysts may be all that are necessary to produce infection in an animal (13, 14, 16, 29) and the inocula used may have contained from 100 to 1,000 cysts. Nonetheless, the successful production of infection in the animal did indicate that viable cysts were present within many of the samples after their removal from exposure to water at different time points.

A surprising result of this study was the loss of viability when *Giardia* cysts were exposed to Minneapolis tap water for as little as 3 days, as compared with the survival of cysts in unprocessed river water. The cysticidal effect of tap water (in a university laboratory) was observed in both seasons, and on a third occasion, a similar result was obtained during the summer by using residential tap water (unpublished observation). It has not been determined exactly what factor(s) present in this processed tap water affects *Giardia* cyst viability, but most likely, it represents the effect of residual chloramine (1987 daily average, 3.4 mg/liter; range, 2.9 to 3.6 mg/liter [*Annual Report for 1987 of the Minneapolis Water Works*, Minneapolis, Minn.]) found in Minneapolis tap water. Outbreaks of giardiasis caused by waterborne *Giardia* spp. have been reported associated with contamination of broken water mains by ruptured sewage lines (10, 11), but our results using *G. muris* cysts suggest that the risk of similar giardiasis outbreaks under water treatment conditions like that of Minneapolis might not exceed 3 to 7 days, especially since *G. muris* cysts have a greater resistance to disinfection agents like chloramine than do *G. lamblia* cysts (18, 30).

In conclusion, *Giardia* cysts were detected by their morphological characteristics in samples exposed to environmental water at every time point examined, even though the viability of cysts suspended in water never exceeded 1 to 3 months. The detection of *Giardia* cysts in surface water used for drinking purposes might be considered to constitute an immediate threat of waterborne giardiasis, but our results indicate that the mere presence of *Giardia* cysts in water did not always correlate with cyst viability as measured by dye exclusion or the ability to infect animals.

ACKNOWLEDGMENTS

We thank Susan Spence, Paul Melchior, Robert Erlandsen, Mary Januschka, LeeAnn Sherlock, and Chris Frethem for their help in collecting samples and technical assistance. We also thank Timothy Hutchison, Minneapolis Water Works, for assistance in the analysis of water samples. We also acknowledge the Limnological Research Center, University of Minnesota, for the use of their water collection equipment and W. J. Bemrick for his critical review of the manuscript.

The research described has been supported by the Minnesota Medical Foundation and in part by the U.S. Environmental Protection Agency through cooperative agreement CR-814622.

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