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RESEARCH PROJECT SUMMARY

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SURVEY OF SURFACE SOURCE WATERS FOR GIARDIA AND CRYPTOSPORIDIUM AND WATER TREATMENT EFFICIENCY EVALUATION

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ABSTRACT

Giardia and Cryptosporidium (shortened to "Crypto") parasites are among the leading identified causes of waterborne disease in the United States. Giardia cysts and Crypto oocysts have been identified in many surface waters throughout the country, but the concentrations in most of New Jersey's (NJ) surface waters, many of which are used as source waters for drinking water treatment plants, are unknown. To provide safe drinking water, treatment plants need to remove Giardia and Crypto from untreated water or reduce their concentrations to safe levels. Peak parasite levels, together with adverse water treatment factors, such as low water temperature, determine the maximum risk of human infection. However, parasite testing is not required by law and is not routinely performed by water treatment plants or regulatory personnel because the methods available are difficult, expensive, time-consuming, and have other drawbacks.

In this project, 15 sites ($\approx 45\%$ of NJ's surface source waters) were selected to represent the full anticipated range of water quality and potential parasite impact in the state. Each site was sampled 10 times over a one year period at varying turbidity levels (final $n=147$). Parasites were isolated and partially purified from 100 gallon samples and examined microscopically. Giardia and Crypto were detected in 23% and 20% of the samples at average concentrations of 2.1 cysts/liter (range 0.4-6.3) and 2.0 oocysts/liter (range 0.3 - 9.8), respectively. These concentrations were similar to those found in an earlier national study. No individual location had unusually high parasite concentrations. Parasite levels did not correlate with any other, more easily measured water quality parameter such as turbidity or coliform bacteria. The treatment effectiveness of two of the treatment facilities was monitored on 9 parasite sampling days by measuring particle count reductions and determining disinfectant (chlorine) concentration and contact time ($C \times t$) inactivation values for Giardia. Source water Giardia levels were reduced following treatment to a health risk-based goal of 0.0007 organisms per 100 liters on all but one occasion when pre-disinfection $C \times t$ values were included with the post-disinfection values. There is currently insufficient health-risk and "field" disinfection data to perform similar treatment efficiency calculations for Crypto.

BACKGROUND

Giardia lamblia (Giardia) and *Cryptosporidium parvum* ("Crypto") are microscopic parasites that can

infect the intestinal tract of humans and animals. These parasites are among the leading identified causative agents of waterborne disease in the U.S. (1). Exposure to these organisms can cause diarrhea, lasting days to

weeks in susceptible persons. Crypto can be life-threatening for individuals with weakened immune systems including persons with AIDS or the AIDS virus, organ transplant or cancer chemotherapy patients, persons on high dose steroid therapy and persons with inherited immunodeficiencies. The two main routes of infection are: 1) "hand-to-mouth" infection following contact with a contaminated person, animal or object; and 2) consuming contaminated water. Infection due to contaminated food has also been observed on occasion.

Giardia and Crypto enter surface waters such as lakes, ponds and reservoirs as environmentally resistant cysts and oocysts (egg-like structures), respectively, in the feces of infected people or animals. Contamination sources include sewage treatment plant effluents, septic tank discharges, and infected pets, farm and wild animals. Fecal material may contaminate waters directly or following storm water runoff. Giardia and Crypto can survive in the environment for weeks or months.

Waterborne infection occurs by consuming contaminated surface water from lakes, ponds or rivers directly, or accidentally during water sport activities. Ground (well) water is usually free of these organisms but occasional contamination has been reported. Treated drinking water has been shown to be a cause of occasional disease outbreaks due to water treatment deficiencies.

In April 1993, after this study was underway, an outbreak of cryptosporidiosis that was traced to Crypto in the drinking water of Milwaukee, Wisconsin caused illness in over 400,000 people with over 4,000 people hospitalized (2). Giardia and Crypto are more resistant to disinfection than viruses or bacteria, including total or fecal coliforms that are routinely monitored as indicator organisms to assess the adequacy of drinking water treatment. Therefore, treated water may contain parasites in the absence of fecal indicator bacteria.

Routine monitoring for Giardia and Crypto is not practical because the available methods are time-consuming and have various limitations. The tests require considerable analytical skill, may not always detect these organisms when present, and cannot reliably identify how many organisms are alive and capable of causing disease. It is not generally practical to monitor treated water for these parasites because

they are typically present at very low concentrations and effective monitoring would require analysis of many high-volume samples (*e.g.* 500 samples over a year; 2,000 liters each (3)).

The U.S. Environmental Protection Agency's (USEPA) Surface Water Treatment Rule (SWTR)(4) stipulates, among other things, that surface water treatment plants remove or inactivate levels of Giardia cysts by 99.9% or more (3 logs) between the untreated intake water and the point of the first customer in the distribution system.¹ Little data exist on the range of concentrations of Giardia cysts and Crypto oocysts in surface waters in NJ. To provide a sufficient level of water treatment at a given plant, it is necessary to know the upper end of the range of concentrations of these organisms in the intake water. Peak parasite levels, together with adverse water treatment factors (*e.g.* low water temperature), determine the maximum risk of human infection. Published research has shown that some treatment plants may be over-treating their water (5). Conversely, under-treatment may result in an unacceptable level of risk to human health.

In February 1993, the Division of Science & Research (DSR), NJDEP, initiated a research project to survey NJ surface source waters for levels of Giardia and Crypto and to investigate the ability of treatment facilities to reduce the levels found in the untreated water to safe levels.

OBJECTIVES

- Analyze 15 surface source waters at or near treatment plant intakes, 10 times each, over the course of the year at varying turbidity levels.
- Correlate the parasite data with conventional water quality parameters, such as turbidity or coliform bacteria.
- Study treatment efficiency at 2 facilities by particle count measurements, and by measuring disinfectant C x t values to determine Giardia cyst inactivation using USEPA data (6).
- Analyze the treatment efficiency data in terms of the treatment level required by the USEPA (4) and a published level calculated not to cause more than 1 infection in 10,000 exposed people per year (3).

PROJECT DESIGN AND METHODS

Fourteen potable water treatment plants that included the full anticipated range of water quality in the state were selected. The participants included large, medium, and small plants in urban, suburban, and rural locations with, comparatively, high, medium, and low quality intake waters. Water quality was defined by turbidity levels, fecal coliform counts, and other parameters. About half the plants have river intakes and half have reservoir or lake intakes. Fifteen surface water intakes were sampled 10 times over the course of one year at varying turbidity levels.² Earlier research showed a significant correlation between parasite concentrations and turbidity levels (5,7).

Methods specified in the proposed federal Information Collection Rule (8) were followed. 100 gallons of untreated intake water were filtered by plant personnel and the filters shipped overnight on ice to the American Water Works Service Company (AWWSC) laboratory in Belleville, IL. *Giardia* cysts and *Crypto* oocysts ("G/C") were collected from the filters, concentrated and partially purified. A portion of the concentrate was reacted with fluorescent dye-tagged antibodies specific for *Giardia* spp. and *Cryptosporidium* spp. and examined with a fluorescence microscope for the presence of fluorescent structures of the appropriate size and shape. Such organisms were labeled **presumptive** G/C. Presumptive G/C were further examined under visible light for the presence of G/C-specific surface and internal structures necessary for viability (potentially infectious). These organisms were labeled **confirmed** G/C. Because only a portion of each processed sample was examined and because the amount examined was different for each sample (depending on the amount of interfering material in the sample and other factors), each sample had its own "detection limit" or parasite level below which the method could not detect parasites, even if present.

Routine intake water quality monitoring data such as coliform bacteria counts, turbidity, pH, and temperature were also collected on each G/C sampling date.

At two of the participating facilities, particle counts ($\geq 3 \mu\text{m}$) were measured in the intake water and at various points in the treatment process to determine particle reductions³. These data, along with intake *Giardia* concentrations and disinfectant $C \times t$ *Giardia* inactivation calculations, were used to determine total

Giardia log reductions on that day.^{3,4} The values obtained were compared to the treatment level required by the USEPA (4) and a published level calculated to result in not more than 1 infection in 10,000 exposed people per year (3) to see if the treatment facilities provided an adequate level of treatment on that sampling day.⁵

RESULTS

Summary statistics on concentrations and detection limits are presented in Table 1. A total of 147 samples were analyzed.⁶ In most samples, *Giardia* or *Crypto* were not found (below [$<$] the detection limit for the sample). The average detection limit for samples in which parasites were not detected was slightly higher than that for samples in which parasites were observed. In addition, the average detection limit for all samples was similar to the average parasite concentration in positive samples. Therefore, the number of samples actually containing parasites was underestimated and actual average parasite concentrations are probably lower, but not higher than the values reported. G or C were found in 35% of the samples. In an earlier study by LeChevallier *et al* surveying 66 facilities in 14 states, 97% samples were positive for G or C (81% for G alone and 87% for C alone) (5). Either parasites occur less frequently in New Jersey compared to some other states or the overall detection frequency has decreased between the time of the earlier study (5) and this one. The geometric mean parasite concentrations found in this study (Table 1) were very similar to those found in the earlier national study (G = 2.8 cysts/liter; C = 2.7 oocysts/liter), however the ranges observed in the earlier study (G = 0.04 - 66 cysts/liter; C = 0.07 - 484 oocysts/liter) were larger than the ranges reported here by about 2 orders of magnitude. There were 34 positive G samples and 30 positive C samples, but only 13 samples in which both G and C were found together. This indicates either 1) that these two organisms do not "track" together and thus may have different environmental sources or 2) reflect the fact that average parasite concentrations were near the average detection limit of the assay. That is, there may have been many samples in which the concentration of one parasite was just high enough to be detected, while the concentration of the other parasite was low enough not to be detected.

The results for individual sites are shown in Table 2. Only one source water was free of detectable parasites

throughout the study (a second source was parasite free on all but one occasion). No individual site had unusually high parasite levels. Due to test sensitivity limitations, it is likely that many negative samples may have contained parasites, but at levels too low to be detected. It would be reasonable to assume that parasites are present at similar levels and frequencies in NJ source waters not tested in this study.

Parasites were detected 2 to 3 times more often in rivers compared to lakes or reservoirs. However, when detected, concentrations in the rivers were slightly lower than in the lakes and reservoirs. Lake and reservoir watersheds are more protected than river watersheds from some, but not all parasite sources.

The number of detected G/C which had confirmed morphologies are shown in Table 3. Parasites with "confirmed" morphologies have a **greater likelihood** of being viable or potentially infectious than those which do not have confirmed morphologies, although the method cannot distinguish viable from non-viable organisms. Few of the detected Giardia had confirmed morphologies (including only 1 of 48 cysts detected in river samples) while over 2/3 of the detected Crypto had confirmed morphologies.

Seasonal differences in the frequency of parasite detections and of parasite concentrations were noted, but there were also seasonal differences in detection limits, so the observed differences may not be real. No easily measured water quality parameter, such as turbidity, coliform bacteria or particle counts, was found to be significantly correlated with G/C concentrations.

Two treatment facilities examined the ability of their treatment process to reduce levels of Giardia in the untreated source water to levels in their treated or finished water that met: 1) the federal 3-log reduction requirement (4); and 2) a level that, based on risk assessment modeling, would cause infection in not more than 1 in 10,000 exposed persons per year. This risk-based level was 0.000007 Giardia cysts per liter (3).

A sample calculation using treatment facility data is shown in Table 4. On a continuous basis, this plant needs to achieve a 3 log reduction to meet federal requirements (as do all surface water plants) and a 5.2 log reduction to meet the 1/10,000 annual infection

level based on risk assessment modeling (7×10^{-6} cysts/liter).

There are a variety of uncertainties underlying the values used in the Giardia risk assessment model, from which the 7×10^{-6} cyst/liter level is derived. Therefore, these calculations do not mean that the federal 3 log reduction requirement is inadequate for providing safe drinking water. Nevertheless, the 3 log requirement may be revised depending upon the data obtained under the forthcoming federal Information Collection Rule (8).

The example shows that, on February 22, 1993, the treatment plant was providing 7.75 logs of Giardia removal/inactivation and so exceeded both the 5.2 log goal as well as the 3 log requirement on that day. Total treatment exceeded 6 logs of Giardia removal/inactivation on all but one of 18 measurements at the two plants studied. The exception was 4 logs of treatment during a cold weather/high water-demand day. There is currently insufficient health-risk and "field" disinfection data to perform similar treatment efficiency calculations for Crypto.

APPLICATIONS

Seasonal effects, such as temperature, have a significant impact on disinfection requirements. Peak parasite levels and adverse treatment factors determine the maximum risk of human infection. To set appropriate treatment level goals, it is imperative for each facility to know the range of G/C concentrations in its intake water. Data from this study can be utilized by all surface water treatment plants in NJ to better determine if their current operating procedures are meeting appropriate treatment goals.

Earlier research has shown that many treatment plants in the U.S. are providing adequate or more-than-adequate water treatment to achieve health-based target Giardia levels in finished water (5). On the other hand, some plants may occasionally provide sub-optimal treatment during adverse treatment conditions (5). Sub-optimal treatment may result in unacceptable human health risks and possible non-compliance with the SWTR. Disease outbreaks, resulting from inadequate treatment, can have adverse financial consequences as demonstrated by the more than \$25 million in pending lawsuits and an estimated \$54 million in other costs (lost wages, hospitalization, and water

utility expenses) resulting from the Milwaukee Crypto outbreak (9).

The results from this research will enable drinking water treatment facilities that derive their water from surface sources to refine treatment goals and to more accurately measure their ability to meet the SWTR Giardia log reduction requirement.

RESEARCH NEEDS

There are no methods presently available, including the one used in this study, that can reliably distinguish live or infectious cysts or oocysts from dead or non-infectious ones. Therefore, the exact percentage of the total parasites detected in this survey, which are of health concern, is not known.

Research is needed on better G/C detection methods, identifying surrogate indicators of parasite concentrations, assessment of new water treatment methods, identification of watershed source inputs, and determination of the infectious potential of the observed parasite cysts/oocysts (10).

REFERENCES

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ENDNOTES

1. Actual monitoring of Giardia is not required. The SWTR Preamble allows treatment plants employing conventional coagulation, sedimentation, and filtration; or direct filtration, slow sand filtration and diatomaceous earth filtration "treatment credits" of 2.5 and 2.0 log Giardia removal respectively with additional log removal/inactivation coming from disinfection (disinfectant concentration/time [C x t] Giardia inactivation).
2. One plant sampled 2 separate river intakes.
3. Particle counting was used as a surrogate for Crypto oocysts, (spherical particles with an average diameter of 4.8 µm [range, 3.3-7.3 µm]) and Giardia cysts, (oval particles with an average width of 8.8 µm [5.8-12.5 µm] and an average length of 12.3 µm [7.3-16.6]).
4. Particles and parasite reductions are expressed in orders of magnitude (*e.g.* 10-fold, 100-fold, etc.). Therefore, data are expressed in their logarithmic or "log" form.
5. There are no health-based target levels for Crypto at this time.
6. One facility switched to a groundwater source during the project and collected 7 rather than 10 samples.

ADDITIONAL INFORMATION

This project was funded by the A-280 Safe Drinking Water Research Fund. A DEP/DSR technical report, describing the results of this project in detail, is available from DSR by calling (609) 292-9692. Further information regarding other research efforts by the division can be obtained by calling (609) 984-6071. DSR Reference No. 95001.

Parasite sampling and intake water quality information was supplied by the participating water treatment facilities. Their cooperation throughout this project is greatly appreciated.

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Table 1. Concentrations of Giardia cysts and Cryptosporidium oocysts in 15 Surface Intake Waters of New Jersey Potable Water Treatment Plants

Parasite ^a Detected	N ^b (% of total)	Geomean ^c	Median ^c	Range ^c
none	96 (65)	< 1.9	< 1.9	< 0.2-< 15.4
G OR C	51 (35)	-	-	-
G	34 (23)	2.1	2.0	0.4-6.3
C	30 (20)	2.0	1.8	0.3-9.8
G AND C	13 (9)	-	-	-

a G = Giardia cysts. C = Cryptosporidium oocysts.

b Total = 147.

c Geomean = geometric mean. Data for presumptive and confirmed G Cysts or C oocysts per liter. For negative samples, less than (<) detection limit.

- Not Applicable.

Table 2. Occurrence of Giardia and Cryptosporidium at Individual Source Water Sites

Site	County	Source Water	No. Samples	Giardia cysts ^a			Cryptosporidium oocysts ^a		
				No. Pos. Samples	Avg.	Range	No. Pos. Samples	Avg.	Range
Burlington	Burlington	Delaware River	10	5	2.0	0.6-5.8	4	1.7	0.6-6.4
Elizabethtown	Somerset	Raritan River	10	4	2.1	1.8-3.0	3	1.0	0.9-1.2
Elizabethtown	"	Millstone River	10	4	1.6	0.9-3.9	4	1.5	0.9-2.4
Franklin	Sussex	Franklin Pond	7	2	3.5	3.1-4.0	3	1.1	0.8-1.5
United Water (Hackensack)	Bergen	Oradell Reservoir	10	0	-	-	2	3.5	3.1-4.0
Hackettstown	Warren	Mine Hill & Burd Reservoirs	10	3	1.2	0.4-4.1	1	9.8	-
Jersey City	Morris	Jersey City Reservoir	10	1	3.3	-	0	-	-
Middlesex	Middlesex	Delaware & Raritan Canal	10	4	1.4	0.8-2.0	4	0.7	0.3-1.8
Newark	Passaic	Charlottesburg Reservoir	10	0	-	-	3	8.2	6.5-9.6
Newton	Sussex	Morris Lake	10	0	-	-	0	-	-
NJ-American: Swimming River	Monmouth	Swimming River Reservoir	10	1	1.3	-	1	1.6	-
NJ-American: Commonwealth	Essex	Reservoirs	10	2	6.2	6.0-6.3	1	6.3	-
NJWSA: Manasquan ^b	Monmouth	Manasquan River	10	2	2.6	2.4-2.8	0	-	-
Passaic Valley	Passaic	Passaic River	10	5	2.7	1.6-4.5	0	-	-
Rahway	Union	Rahway River	10	1	1.0	-	4	3.3	1.8-4.9

a Average (geometric mean) and range per liter of untreated water.

b New Jersey Water Supply Authority.

Table 3. Microscopic observations of Giardia cysts and Crypto oocysts

Parasite	Total n	Number (percent) Observed with:	
Giardia cysts	73	0-1 of 3 internal structures ^a	2-3 of 3 internal structures ("confirmed") ^b
		62 (85%)	11 ^c (15%)
Cryptosporidium oocysts	48	no sporozoites	from 1 to 4 sporozoites ("confirmed")
		15 (31%)	33 (69%)

a Internal structures include nuclei, median bodies, and axenomes.

b Parasites with "confirmed" morphologies have a **greater likelihood** of being viable or potentially infectious than those which do not have confirmed morphologies, although the method cannot distinguish viable from non-viable organisms.

c 5 (7%) also displayed a peritrophic space considered by some scientists as indicative of viability.

Table 4. Example Giardia cyst removal and inactivation at site #10.

Giardia Concentration	Cysts per liter
In untreated water ^a	1.19
Concentration needed in finished water to meet the SWTR ^b (3 log reduction)	0.0019
Calculated treated water concentration causing infection in $\leq 1/10,000$ people/year ^c (= 5.2 log reduction)	0.000007
Level of treatment achieved on 2/22/93:	Log Reduction
Particle Removal ^d	3.02
Inactivation (disinfection) ^e	
Pre-disinfection	3.50
Clearwell (post or final disinfection)	<u>1.23</u>
Total	4.73
Total	<u>7.75</u>

a Value is the 90th percentile value of the range of Giardia concentrations or detection limits for the 10 samples taken at site 10. Unadjusted value of 3.1 cysts/liter was corrected for method recovery efficiency (median = 39%) and percent of all cysts in this study with confirmed morphology (15%).

b USEPA Surface Water Treatment Rule requirement (4).

c Risk Assessment model-derived value (3).

d Difference between concentration of particles (3 μm or greater) in untreated and finished or treated water.

e Values from Giardia inactivation tables (6) and based on water temperature and chlorine concentrations and contact times (C x t) on the day indicated.