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Project Summary

Virus Removal During Conventional Drinking Water Treatment

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The reduction of enteroviruses and rotaviruses was studied at a full scale 205 mgd water treatment plant involving chemical flocculation, sand filtration, and chlorination. Reduction of enteroviruses and rotaviruses averaged 81% and 93%, respectively, for the complete treatment process. The greatest reduction of enteroviruses occurred during pre-chlorination/flocculation and filtration, and reduction of rotaviruses occurred during pre-chlorination/clarification and final chlorination. Enteroviruses or rotaviruses occurred in 24% of the finished water samples containing levels of free chlorine (>0.2 mg/l), and meeting coliform bacteria (1/100 ml) and turbidity (1 NTU) standards. The results of this study indicate that finished water having measurable levels of free residual chlorine and meeting standards for coliform bacteria and turbidity cannot be assumed to be virus free.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Since the turn of the century, it has been known that there is a significant risk of contracting infectious disease from the ingestion of sewage-contaminated water and food. The spread of enteric bacterial disease by this route has been well controlled by the widespread application of bacterial standards and modern treatment processes for drinking water and sewage.

The isolation of viruses from treated drinking water has raised concerns that water treatment methods may not always adequately insure the removal of viruses from water designated for human consumption. Because viruses have been isolated from drinking water that met recommended levels of coliform bacteria, chlorine and turbidity,[1,2,3] serious questions have been raised about the adequacy of bacterial standards in judging the sanitary quality of water relative to its potential for transmitting viral disease.

The majority of processes used to treat sources of potable water are capable of reducing virus numbers. However, enteric viruses have been shown to be less effectively removed or inactivated than indicator bacteria by many treatment processes, and much of the available information on the removal of enteric viruses by water treatment processes is based on laboratory studies with members of the enterovirus group (particularly, poliovirus type 1) and a few selected coliphages. Not all enteric viruses are likely to be removed with equal and high efficiency by a given water process. Laboratory studies have shown that poliovirus is more effectively removed from water by alum flocculation and from sewage by adsorption to sludge flocs than rotavirus. Likewise, under field conditions at an activated sludge sewage treatment plant, enteroviruses appeared to be more effectively removed than were rotaviruses. It is also recognized that naturally occurring viruses may be more resistant to chlorination than prototype laboratory strains.

Few studies have been done on the removal of naturally-occurring enteroviruses by actual drinking water treatment plants under field conditions. A previous report[3] describing research in which enteroviruses and rotaviruses were isolated from treated drinking water in a distribution system and at a water treatment plant initiated a more extensive investigation at the same site. This study reports on the occurrence of enteric viruses within a full-scale water treatment plant after treatment processes including clarification, filtration, and chlorination.

Procedure

The site in this study was a 205 mgd water treatment plant supplied with raw water from a river and conveyed approximately 17 km by canal via two pumping stations. The river is fed by a shallow lake located in a watershed with a human population of 4 to 8 million, where untreated wastewater is discharged.

Each plant has a treatment sequence consisting of chemical addition (liquid alum and catfloc or superfloc) followed by hydraulic mixing, flocculation, clarification with pre- and post-chlorination, filtration through rapid sand filters or automatic valveless sand filters and final chlorination. Samples collected included raw or intake water, water post-clarification, water post-filtration, and finished water from three of the five plants. Four trips to the plant were made over a two-year period and 20 to 40 samples were collected during each trip.

For enteric virus collection and concentration, samples of 9.8 to 756 liters were collected using the adsorption-elution method and 1 MDS Virosorb filters [AMF CUNO, Meriden, CT]. Primary filter eluates were reconcentrated from a volume of 1 L to approximately 36 ml by an organic flocculation method. All samples for enterovirus analysis were inoculated onto monolayers of BGM cells, 2 ml/75 cm² plastic flask, overlaid with maintenance medium and observed for cytopathic effects (CPE) for a period of 21 days. Those samples positive for CPE were passed a second time in the BGM cell line and then plaqued as a final confirmatory test using an agar overlay method. Subsequently, virus isolates were identified by serum neutralization tests using the Lim-Melnick enterovirus Antibody Serum Pools. Rotaviruses were detected by using the immuno-fluorescent assay described by Smith and Gerba.[4]

One-L grab samples and virus samples were taken simultaneously. Bacterial densities, standard plate count bacteria, total and fecal coliform bacteria, and fecal streptococci bacteria, were determined using membrane filtration. Bacteriophage concentrations were determined in the grab samples as well as in each 1 MDS filter eluate, before freezing, using *Escherichia coli* Hfr (ATCC 15597) as the host bacterium. Physical-chemical measurements of the water included total and free chlorine levels, pH and turbidity.

Results and Discussion

The biological and physico-chemical characteristics of the water after the treatment steps were examined over a two-year period. Four sampling periods were chosen, three during the dry season (3/82, 1/83 and 4/83) when the plant was operating more optimally and one during the rainy season (7/82) when the plant could not produce water meeting minimum coliform and turbidity standards.

The mean values for turbidities, bacteria and viruses were calculated for each sampling trip for the dry season only. In general, the total plate count bacteria were found to be the most numerous, ranging from 9/ml in the finished water to 465/ml in the raw water, followed by the fecal streptococci, (20 to 4453/100 ml), enterococci (6 to 1165/100 ml), total coliforms (15 to 216/100 ml) and fecal coliforms (1 to 66/100 ml). Coliphage concentrations ranged from 1/L in the finished water to 1769/L in the raw water.

Table 1 summarizes the data on enteric virus isolation from all the samples during the dry season. Viruses were recovered in 68% of the raw water samples and after clarification and filtration; 50% and 37% of the samples were positive for viruses, respectively. Viruses were detected in 20% of the finished water samples. It was found that eight (24%) of the finished water samples that contained free chlorine levels of greater than 0.2 mg/L and met turbidity and bacterial standards were also positive for enteric viruses.

Average percent reductions for all water quality parameters studied were determined after each step in the water treatment processes (Table 2). The major reduction in bacteria and coliphage, (90% to 99%) came after pre-chlorination/clarification with slight decreases during the other treatment steps. The initial reduction of microorganisms during the treatment process may be due to the addition of chlorine, although adsorption to the floc during clarification undoubtedly is capable of removing large numbers of organisms. The greatest reduction in turbidity occurred during the clarification step and averaged 87% after complete treatment.

The greatest removal of enteroviruses appeared to occur during pre-chlorination/clarification and filtration steps. The major reduction of rotaviruses occurred after pre-chlorination/clarification and post-chlorination of the finished water. Of all the groups of microorganisms studied, enteric viruses were the least effectively removed.

From this study and others[1,2,3], it appears that enteric viruses can occur at detectable levels in finished drinking water meeting current coliform, turbidity, standards and containing levels of free chlorine (0.2 mg/L). The isolation of human enteric viruses in these waters is not a condemnation of conventional drinking water treatment but an indication that water quality parameters currently accepted to ensure the production of microbially safe water do not necessarily ensure the absence of enteric viruses.

Although the plant was able to produce water with a turbidity of 1 NTU and less than one total coliform per ml, major operational and design deficiencies were apparent. The most serious problem was in the filters where cracking, mudballs, and sand boils were observed, indicating shortcircuiting. The plant may have been operating near or above its design capacity (hydraulically overloaded) but the extent of the problem could not be determined due to the lack of metering. The chlorine contact time could not be determined; however, operators reported it as less than 30 minutes at peak flows dropping as low as 15 minutes. This could explain the occurrence of enteric viruses in the finished water. Still it is significant that these deficiencies were not always reflected by the quality of the finished water during the dry season. It may be that, in marginally operated water treatment plants or plants with a heavily contaminated raw water source, measureable levels of enteric viruses are able to penetrate the treatment process, even when current water quality standards are achieved.

Table 1. *Summary of Enteric Virus Isolation from All Samples[a] After Successive Drinking Water Treatment Processes*

Site	Raw		Clarified		Filtered		Finished		Finished: Samples Meeting[b] Standards	
Number of Samples Collected	19		14		8		54		33	
Virus	Number Positive	%	Number Positive	%	Number Positive	%	Number Positive	%	Number Positive	%
Enterovirus	9	47	4	29	0	0	5	9	4	12
Rotavirus	9	47	5	36	3	37	7	13	5	15
Both Entero & Rotaviruses[c]	5	26	2	10	0	0	1	2	1	3
Total Enteric Viruses	13	68	7	50	3	37	11	20	8	24

[a] = All samples collected during the dry season only.
[b] = Number of finished water samples positive for virus which had free chlorine residual and met U.S. turbidity, and bacterial standards.
[c] = Number of samples positive for both Entero- and Rotaviruses.

Table 2. *Average Percent Reductions of Turbidity, Bacteria and Viruses After Successive Drinking Water Treatments*

From Raw to Site	Date	Collection	Turb. NTU	Total Plate Count	Bacteria: Total Coliforms	Bacteria: Fecal Coliforms	Bacteria: Fecal Strep	Bacteria: Enterococci	Coliphage (Direct)	Coliphage (Concentrate)	Enterovirus CPE	Rotavirus
Prechlorinated/ Clarified	3/82	1	11	47.5	100.0	99.5	99.5	ND[b]	28.5	99.0	25	95
	1/83	3	0[a]	89.1	94.3	100	92.6	97.3	97.0	99.91	78	0
Grand Mean			4	78.9	98.9	97.1	97.2	97.3	90.4	99.7	55	61
Filtered	1/83	3	73	98.9	98.5	100	99.7	99.8	99.8	99.9	100	0
Finished	3/82	1	81	91.7	100	100	99.7	ND	100	99.8	45	99
	1/83	3	76	98.6	98.6	100	99.5	99.5	99.9	99.9	92	0
	4/83	4	90	98.6	98.9	99.3	99.7	ND	99.9	99.8	100	93
Grand Mean			87	98.1	99.3	98.5	99.5	99.5	99.9	99.9	81	93

[a] = No removal.
[b] = Not determined.
[c] = Mean reductions determined using mean counts weighted by number of samples per trip.

Conclusions

1. Both enteroviruses and rotaviruses could be isolated from finished drinking water containing chlorine levels of >0.2 mg/L and meeting coliform bacteria (1/100 ml) and turbidity (1 NTU) standards.
2. Enterovirus and rotavirus removal averaged 81% and 93%, respectively, for the complete treatment process involving prechlorination/flocculation, sand filtration, and final chlorination.
3. The complete water treatment process was more effective in the removal of turbidity, coliform, fecal coliform, fecal streptococci, standard plate count bacteria, and coliphage than enteric viruses.
4. Pilot plant studies as well as field studies should be conducted in order to determine the occurrence and significance of enteric viruses after water treatment processes and to evaluate coliphages as an indicator system for animal viruses as well as other water quality parameters.

Recommendations

1. Studies should be conducted to determine the efficiency of human rotavirus removal by drinking water treatment processes.
2. Better methods should be developed for the detection and concentration of coliphage from water.
3. Longer term studies should be conducted on the occurrence of enteroviruses and rotaviruses in treated drinking water taking advantage of the continual development of detection methods. This

would allow the development of a larger data base which could be used to determine if stronger correlations exist between the presence of coliphage, total plate count bacteria and enteric viruses.

4. Research should be conducted on water quality and operational treatment conditions that will assure essentially complete removal of viruses.

References

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The complete report, entitled "Virus Removal During Conventional Drinking Water Treatment," (Order No. PB 85-227 510/AS; Cost: $10.00, subject to change) will be available only from:

National Technical Information Service
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