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Detection of Enteric Viruses in Treated Drinking Water

BRUCE H. KESWICK,[1] CHARLES P. GERBA,[2]* HERBERT L. DuPONT,[1] AND JOAN B. ROSE[2]

Program in Infectious Diseases and Clinical Microbiology, University of Texas Medical School, Houston, Texas 77030,[1] and Departments of Microbiology and Immunology and Nutrition and Food Science, University of Arizona, Tucson, Arizona 85721[2]

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The occurrence of viruses in conventionally treated drinking water derived from a heavily polluted source was evaluated by collecting and analyzing 38 large-volume (65- to 756-liter) samples of water from a 9 m^3/s (205×10^6 gallons [776×10^6 liters] per day) water treatment plant. Samples of raw, clarified, filtered, and chlorinated finished water were concentrated by using the filter adsorption-elution technique. Of 23 samples of finished water, 19 (83%) contained viruses. None of the nine finished water samples collected during the dry season contained detectable total coliform bacteria. Seven of nine finished water samples collected during the dry season met turbidity, total coliform bacteria, and total residual chlorine standards. Of these, four contained virus. During the dry season the percent removals were 25 to 93% for enteric viruses, 89 to 100% for bacteria, and 81% for turbidity. During the rainy season the percent removals were 0 to 43% for enteric viruses, 80 to 96% for bacteria, and 63% for turbidity. None of the 14 finished water samples collected during the rainy season met turbidity standards, and all contained rotaviruses or enteroviruses.

The isolation of viruses from treated drinking water (2, 3, 10, 12) has raised concerns that water treatment methods may not always adequately remove viruses from water designated for human consumption. In fact, viruses have been isolated from drinking water which met acceptable coliform, chlorine, and turbidity limits (T. R. Deetz, E. M. Smith, S. M. Goyal, C. P. Gerba, J. J. Vollet, L. Tsain, H. L. DuPont, and B. H. Keswick, Water Res., in press). The question is raised as to whether current drinking water standards ensure safe drinking water. In a previous report (Deetz et al., in press), we described the isolation of enteroviruses and rotaviruses from treated drinking water in a distribution system and at a water treatment plant. This paper reports the results of a more extensive investigation of the removal of viruses by water treatment processes, including clarification, filtration, and chlorination, at a full-scale water treatment plant.

MATERIALS AND METHODS

Water treatment plant. A 9 m^3/s (205×10^6 gallons [776×10^6 liters] per day) water treatment plant was selected for study based on our previous results indicating that the raw water source contained high amounts of viruses. Raw water is withdrawn from a river and conveyed ca. 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 4×10^6 to 8×10^6, and untreated water and wastewater are discharged into the watershed. The treatment facility consists of five separate plants constructed over a 20-year period from 1955 to 1975. Each plant has a treatment sequence consisting of chemical addition (liquid alum and Catfloc or Superfloc [Calgon Corp., Pittsburgh, Pa.]), followed by hydraulic mixing, flocculation, clarification with pre- and postchlorination, filtration either through rapid sand filters or automatic valveless sand filters, and final chlorination. All samples were collected from three of the plants.

Water sample collection. Samples of 9.8 to 756 liters were collected by using 1MDS Virosorb filters (AMF CUNO, Meriden, Conn.), which eliminate the necessity to precondition the water. An electric 1-horsepower centrifugal pump was used to collect samples which could not be collected directly from taps. Where necessary, in-line injectors (DEMA, St. Louis, Mo.) were used to inject sodium thiosulfate into water to neutralize the chlorine before passage through the virus-adsorbing filters (11). Samples were collected at the canal intake delivering water to the plant, after clarification, after filtration, and after final chlorination. On each day that samples were collected, finished water samples were always collected first and intake water samples were always collected last. Separate filter housings and hoses were used for each sampling location. The filter housings were disinfected by soaking for 30 min in buckets filled with water containing more than 5 mg of free chlorine per liter. Hoses and pumps were disinfected by pumping water containing more than 5 mg of free chlorine per liter through them. Concurrent 1-liter grab samples were collected for bacterial, bacteriophage, and turbidity analyses.

* Corresponding author.

Elution and reconcentration procedures. Adsorbed viruses were eluted by passage of 1 liter of 3% beef extract (Scott Laboratories, Fiskeville, R.I.), (pH 9.5) into the filter housing. The pH of the eluent solution was adjusted just before use by the addition of 1 N NaOH. Once the housing was filled, the beef extract solution was allowed to remain in the housing for 2 to 5 min and then was forced out of the housing with air. The pH of the eluate was adjusted to 7.0 to 7.5 by the addition of 1 N HCl. Samples were frozen for shipment to the laboratory at the University of Arizona. Thawed samples were reconcentrated to an average volume of 36 ml by the organic flocculation method (7), and the final concentrate was divided into three equal volumes, one for enterovirus analysis, one for rotavirus analysis, and one to be stored for future use.

Virus detection. All samples were processed under code. Samples for enterovirus analysis (2 ml) were inoculated onto monolayers of BGM cells in 75-cm^2 plastic flasks, overlaid with maintenance medium, and observed for cytopathic effect (CPE) for a period of 21 days. Samples which did not produce CPE were passed an additional two times on monolayers of BGM cells and observed for CPE to ensure that no positive samples were missed. Samples positive for CPE were confirmed by passage to a second BGM monolayer, and after development of CPE, they were plaqued by using an agar overlay method (9).

TABLE 1. Water quality in samples collected after successive drinking water treatment processes (dry season)

Treatment process	Sample no.	Date (1982)	Vol (liters)	pH	Total chlorine (mg/liter)	Free chlorine (mg/liter)	Turbidity (NTU)
None (raw)	5	3/2	76	7.6	0	0	ND[a]
	6	3/2	67	7.6	0	0	ND
	11	3/3	65	8.0	0	0	6.1
	12	3/3	111	8.0	0	0	6.1
	19	3/4	77	ND	0	0	7.1
Clarified	3	3/2	128	7.8	1.4	0.3	5.5
	4	3/2	111	7.8	1.5	0.4	6.1
	9	3/3	90	7.9	1.7	1.4	6.7
	10	3/3	116	7.9	1.7	1.5	6.7
	18	3/4	87	7.9	1.9	1.1	3.3
Finished[b]	1	3/2	525	6.8	7.5	1.0	0.73
	2	3/2	756	6.8	6.5	1.0	0.84
	7	3/3	416	7.8	ND	0.75	0.64
	8	3/3	416	7.8	ND	0.75	0.64
	15	3/4	756	7.7	1.9	0.5	0.95
	13	3/3	513	8.1	2.7	2.2	2.9
	14	3/3	549	8.1	2.7	2.0	2.9
	16	3/4	626	7.9	2.6	2.5	0.65
	17	3/4	691	7.9	2.5	2.1	0.65

[a] ND, Not done.
[b] Finished water was treated by clarification, sand filtration, and chlorination.

Rotavirus was detected by inoculating the samples onto cultures of MA-104 cells (13) in Lab-Tek glass chamber slides (Miles Scientific, Div. of Miles Laboratories, Inc., Naperville, Ill.) in the presence of medium containing trypsin (0.5 μg/ml, Sigma type IX; Sigma Chemical Co., St. Louis, Mo.). Samples which showed toxicity (1) were filtered through 0.45-μm cellulose nitrate filters (Millipore Corp., Bedford, Mass.) or diluted 1:5 or 1:10. After 24 h of incubation at 38°C, the medium was aspirated off, the chamber was removed, and the monolayers were fixed in −20°C methanol for 5 min. The slides were then stained for the fluorescence assay described by Smith and Gerba (13). Primary guinea pig or rabbit antisera directed against human rotavirus was obtained from the National Institute of Allergy and Infectious Diseases reference reagents and from DAKO (Subsidiary of Accurate Chemical & Scientific Corp., Westbury, N.Y.). Secondary antisera conjugated with fluorescein isothiocyanate was obtained from Miles Laboratories (Elkhart, Ind.).

Bacteriophages in both grab samples and beef extract concentrates were analyzed by plaque assay on *Escherichia coli* Hfr host bacteria (4).

Bacterial measurements. Grab samples were tested by the membrane filter method (1) with Nalge Nutrient Pad kits (Rochester, N.Y.) for standard plate count, total coliforms, fecal coliforms, and fecal streptococci.

Water quality measurements. The pH of the water collected was measured on site with a portable pH meter. Samples for turbidity testing were frozen and returned to the laboratory where they were read on a Hach turbidity meter (Hach Chemical, Loveland, Colo.). Residual chlorine was measured with a DPD colorimetric kit (Hach Chemical).

Quality assurance. Stool specimens or rectal swabs were collected from each of the laboratory personnel coming in contact with the samples. All personnel wore gloves when handling filters or samples throughout the course of analysis. The specimens were processed and inoculated onto cell cultures in designated areas physically separated from areas where laboratory strains of viruses are in use. Heavily chlorinated tap water (378 liters) collected in Tucson, Ariz., was also examined to control for false-positive specimens.

RESULTS

The first sampling trip was made during the dry season (March 1982); 19 samples were collected. The sample volumes ranged from 65 to 756 liters (Table 1). Five samples were collected from raw water, five after the clarification process, and nine after final chlorination (finished water). Overall, 12 of the samples were positive for enterovirus and 10 were positive for rotavirus. Significantly, five finished water samples contained enterovirus and two contained rotavirus. None of the finished water samples contained any total coliform bacteria, but fecal streptococci were detected in six of six samples examined. The total plate count bacteria ranged from 1 to 110 CFU/100 ml, with a mean of 28. The mean pH value of the finished water samples was 7.6, and the mean total chlorine and free chlorine contents were 3.7 and 1.42 mg/liter, respectively (Tables 1 and 2).

The five samples of raw water had a mean total plate count of 255 CFU/ml and a mean total coliform count of 870 CFU/100 ml (Table 3). The mean turbidity was 6.4 nephelometric turbidity units (NTU), and the mean pH was 7.6. Four of the five samples were positive for enterovirus and five of five were positive for rotavirus.

Samples collected after the clarification process contained a mean total plate count bacteria of 194 CFU/ml, a mean pH of 7.8, and a mean turbidity of 5.6 NTU. Three of five samples were positive for enterovirus and three of five were positive for rotavirus.

The percent reduction effected by each step in the water treatment process was calculated from the mean measurement of turbidity, total plate count bacteria, total coliform bacteria, fecal streptococcal bacteria, enteroviruses, coliphages, and rotaviruses (Table 3). It should be noted that the mean recovery was higher and the percent reduction was lower for finished water than for filtered water. Since these

TABLE 2. Isolation of bacteria and viruses after successive drinking water treatment processes (dry season)

Treatment process	Sample no.	Bacteria: Total plate count (CFU/ml)	Bacteria: Total coliforms (CFU/100 ml)	Bacteria: Fecal coliforms (CFU/100 ml)	Bacteria: Fecal streptococci (CFU/100 ml)	Viruses: No. of coliphages/10 ml (direct)[a]	Viruses: No. of coliphages/1,000 ml (concentrate)	Viruses: Enterovirus cultures[b]	Viruses: No. of rotaviruses/100 liters
None (raw)	5	340	240	140	3,400	14	12,700	1/3	268
	6	340	240	140	3,400	ND[c]	ND	3/5	1,202
	11	170	1,500	140	1,820	0	21,000	3/3	1,163
	12	170	1,500	140	1,820	ND	17,300	0/4	324
	19	ND	ND	910	ND	21	292,700	3/3	94
Clarified	3	530	0	0	120	2	25,200	4/4	148
	4	120	0	1	27	3	0	1/5	36
	9	63	0	0	0	17	0	3/3	0
	10	63	0	0	0	0	300	0/4	586
	18	ND	0	ND	ND	0	0	0/7	0
Finished[d]	1	21	0	0	1	0	0	5/5	233
	2	110	0	0	27	0	100	0/5	0
	7	16	0	0	16	0	0	3/3	0
	8	16	0	0	16	0	0	1/5	126
	15	ND	0	ND	ND	0	0	0/5	0
	13	1	0	0	3	0	0	3/3	0
	14	1	0	0	3	ND	0	0/4	0
	16	ND	0	ND	ND	0	0	4/4	0
	17	ND	0	ND	ND	ND	0	0/5	0

[a] Water was plated directly for coliphages on *E. coli* Hfr host bacteria.
[b] Enteroviruses were detected by the production of CPE in monolayer cultures of BGM cells contained in 75-cm^2 flasks. Number positive/number tested.
[c] ND, Not done.
[d] Finished water was treated by clarification, sand filtration, and chlorination.

samples were not collected temporally, water quality fluctuations may have been responsible for these results.

Several factors may affect water quality and so water treatment (5). In the study area, raw water quality decreases during the rainy season, as turbidity increases due to storm runoff. To determine the effects of decreased water quality on virus removal by water treatment, the second sampling trip was conducted during the rainy season (July 1982). As indicated by the turbidity and bacterial measurements, a decrease in water quality was reflected by an increased frequency of virus isolation in samples collected during July 1982 (Tables 4 and 5). The mean counts of coliphages and rotaviruses were greatly increased over those obtained from dry season samples. In nine finished water samples, the mean turbidity was 9.6 NTU, the mean pH was 6.6, the mean total residual chlorine was 8.8 mg/liter, and the mean free chlorine was 0.6 mg/liter. Means for total plate count bacteria (716), total coliforms (5,140), fecal coliforms (2), and fecal streptococci (3,726) were also higher than in the dry season. Data for raw water and clarified water are also presented. The percent removals were calculated as above (Table 3).

DISCUSSION

The analysis of samples collected during the dry season (Tables 1 and 2) confirms our earlier findings (Deetz et al., in press) that viruses may be present in water which meets acceptable limits of turbidity (<1.0 NTU), residual chlorine

TABLE 3. Efficiency of virus removal by water treatment processes

Season and treatment process	Turbidity (NTU)	Bacteria[a]: Total plate count (CFU/ml)	Bacteria[a]: Total coliforms (CFU/100 ml)	Bacteria[a]: Fecal streptococci (CFU/100 ml)	Viruses[a]: Rotavirus[b]	Viruses[a]: Enterovirus[c]	Viruses[a]: Phage[d]
Dry							
None (raw)	6.4	255	870	2,610	610	55	11
Clarified	5.6 (12.5)	194 (23.9)	0 (100)	36 (98.6)	154 (74.3)	35 (36.8)	4 (63.6)
Finished[e]	1.2 (81.3)	28 (89.0)	0 (100)	11 (99.6)	40 (93.5)	41 (25.0)	0 (100)
Rainy							
None (raw)	26	3,610	46,500	1.1×10^5	1,745	7	47
Clarified	10 (61.5)	76 (97.8)	740 (98.4)	510 (99.5)	3,417 (0)	29 (0)	16 (65.7)
Filtered	6.8 (73.8)	195 (94.5)	748 (99.6)	1,000 (99.0)	342 (80.4)	15 (0)	3 (93.6)
Finished[e]	9.6 (63.1)	716 (80.2)	5,140 (88.9)	3,726 (96.6)	990 (43.3)	7 (0)	4 (91.4)

[a] Mean recovery and (in parentheses) percent reduction from that in raw water.
[b] Rotavirus infectious foci per 100 liters.
[c] Percent cultures positive for enterovirus CPE.
[d] Coliphage PFU by direct assay of sample.
[e] Finished water was treated by clarification, sand filtration, and chlorination.

TABLE 4. Water quality in samples collected after successive drinking water treatment processes (rainy season)

Treatment process	Sample no.	Date (1982)	Vol (liters)	pH	Total chlorine (mg/liter)	Free chlorine (mg/liter)	Turbidity (NTU)
None (raw)	26	7/19	15	7.6	0	0	23
	31	7/20	9.8	7.2	0	8	29
Clarified	25	7/19	65	6.6	5.0	0.1	17
	30	7/20	37.4	6.4	0	0.1	3.4
Filtered	29	7/20	378	6.8	8.5	0.8	8.6
	36	7/21	340	6.6	>10	1	6.5
	37	7/21	340	6.5	>10	0.6	7.8
	34	7/21	378	6.4	>10	>0.1	1.5
	35	7/21	359	6.9	>10	0.1	10
Finished[a]	24	7/19	412	6.9	>10	0.5	6
	38	7/22	378	6.8	6.5	0	13
	39	7/22	376	6.7	>10	0.4	8.9
	22	7/19	375	6.0	>10	1.1	8.6
	23	7/19	246	6.2	>10	1.2	15
	27	7/20	247	6.7	>10	0.8	15
	28	7/20	268	6.8	>10	0.6	8.5
	32	7/21	378	6.7	>10	1.4	7.2
	33	7/21	467	6.7	8.0	0.7	4.5

[a] Finished water was treated by clarification, sand filtration, and chlorination.

(>0.2 mg/liter), and total coliform bacteria (<1 CFU/100 ml). Seven of nine finished water samples collected met these criteria; four of these contained either enteroviruses or rotaviruses. Importantly, none of the samples containing viruses had detectable total coliform bacteria. In contrast, four of four samples containing viruses also contained fecal streptococci, and three of these met the criteria for turbidity, total chlorine, and total coliforms.

We were also interested in the ability of coliphages to serve as indicators of the animal virus content of water. The results indicate that direct plating of water for coliphages did not reflect the animal virus content of finished water. Furthermore, in several samples phages were detected in the concentrates but not by direct plating of the sample. A concentration procedure may be necessary for coliphages to serve a useful function as indicators.

Since rotavirus is a major cause of gastroenteritis and may be waterborne (13), the ability of the water treatment process to remove rotavirus was of major interest. In this study, ca. 94% of the rotaviruses present in raw water were removed by the treatment process during the dry season. As expected, during the rainy season, when the quality of the raw water declined, so did the removal of rotavirus. It is likely that adsorption to particulates which were not removed during the clarification and filtration steps protected the rotaviruses from final chlorination (6). Further studies on the susceptibility of human and animal types of rotavirus to the action of disinfectants and the manipulation of water treatment processes for their enhanced removal are indicated.

Five of nine (56%) of the finished water samples collected during the dry season contained enteroviruses. In only one of these samples did the turbidity exceed 1 NTU; thus, high turbidity alone may not account for the failure to remove viruses during water treatment. Furthermore, it should be noted that the lower mean turbidity and bacterial removal detected in finished water during the rainy season (Table 3) is likely due to the fact that the samples were not collected temporally, and so short-term fluctuations in water quality were not accounted for. Statistical analysis of the relationship of water quality measurement to the numbers of enteroviruses and rotaviruses recovered awaits the results of analyses on all 120 samples which have been collected in this study. Enteroviruses recovered from these samples have not yet been identified due to the recent inavailability of the Lim-Benyesh-Melnick antisera pools. Further efforts are being made to quantify the numbers of enteroviruses recovered and to identify the rotavirus isolates as either human or animal strains.

A further difficulty encountered has been the coconcentration of material which interferes with the assay of viruses in cell culture. This may have reduced the rate of recovery of enteroviruses in the raw water during the rainy season because of poor water quality. Since the assays for rotaviruses and enteroviruses are conducted under different conditions, this also may explain the differences in recovery of enteroviruses and rotaviruses during the rainy season. Alternatively, there may have been more rotaviruses in the water, as the rainy season appears to coincide with the peak period of rotavirus activity in the area (8; Deetz et al., in press). Preliminary testing indicated that the filtration process used to remove toxicity did not greatly affect the ability to detect viruses in these samples. However, this could not be determined for each sample since many were too toxic to be assayed unfiltered.

Drinking water treatment should and usually does produce microbiologically safe drinking water. As evidenced by the isolation of viruses from treated drinking water (3, 10), this is not always the case. Upon completion of this project we hope to be able to provide a prediction of the conditions, as measured by water quality parameters, under which viruses may survive water treatment. This study confirms that acceptable water quality measurements of turbidity, total residual chlorine, and total coliform bacteria do not necessarily reflect the virus content of treated drinking water. However, since not all finished water samples met each of these criteria, it is likely that operational difficulties were sometimes encountered as water quality fluctuated, indicating that the water treatment process in general is sound, but that under certain conditions viruses may survive treatment.

TABLE 5. Isolation of bacteria and viruses after successive drinking water treatment processes (rainy season)

Treatment process	Sample no.	Bacteria				Viruses			
		Total plate count (CFU/ml)	Total coliforms (CFU/100 ml)	Fecal coliforms (CFU/ 100 ml)	Fecal streptococci (CFU/100 ml)	No. of coliphages/10 ml (direct)[a]	No. of coliphages/ 1,000 ml (concentrate)	Enterovirus cultures[b]	No. of rotaviruses/100 liters
None (raw)	26	6,200	73,000	9,000	132,000	76	173,000	1/9	3,491
	31	1,020	20,000	0	89,000	18	110,000	0/5	0
Clarified	25	69	200	0	300	1	0	0/4	930
	30	82	1,280	0	720	31	0	2/3	5,904
Filtered	29	170	250	640	2,800	8	100	0/5	171
	36	620	120	0	160	1	0	2/3	463
	37	43	42	0	30	3	2,000	0/4	88
	34	100	30	0	2,000	1	0	0/3	585
	35	43	300	0	10	6	800	1/5	404
Finished[c]	24	53	70	10	540	9	13,000	0/4	10,448
	38	ND[d]	ND	ND	ND	4	49,000	0/5	69
	39	ND	ND	ND	ND	3	50,000	0/5	106
	22	>200	>2,000	0	640	4	0	1/4	243
	23	>200	>2,000	0	1,152	4	300	0/4	332
	27	>200	>2,000	ND	>200	6	0	0/5	57
	28	240	>2,000	5	>2,000	4	100	0/3	284
	32	1,230	15,300	0	16,100	10	0	0/4	157
	33	1,340	50	0	200	2	0	0/3	459

[a] Water was plated directly for coliphages on *E. coli* Hfr host bacteria.
[b] Enteroviruses were detected by the production of CPE in monolayer cultures of BGM cells contained in 75-cm^2 flasks. Number positive/number tested.
[c] Finished water was treated by clarification, sand filtration, and chlorination.
[d] ND, Not done.

Common operational factors which may have contributed to these results include inadequate floc formation, floc breakdown, and filter overloading. These conditions can lead to increased amounts of particulates in finished water which can render terminal disinfection ineffective (6). The prediction of conditions which favor the survival of viruses in treated drinking water should enable improvements to be made in design and operation of water treatment facilities which will increase the likelihood that finished water is virus safe.

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