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Elimination of Viruses and Indicator Bacteria at Each Step of Treatment during Preparation of Drinking Water at Seven Water Treatment Plants

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Seven drinking water treatment plants were sampled twice a month for 12 months to evaluate the removal of indicator bacteria and cytopathogenic enteric viruses. Samples were obtained at each level of treatment: raw water, postchlorination, postsedimentation, postfiltration, postozonation, and finished (tap) water. Raw water quality was usually poor, with total coliform counts exceeding 105 to 106 CFU/liter and the average virus count in raw water of 3.3 most probable number of cytopathogenic units (MPNCU)/liter; several samples contained more than 100 MPNCU/liter. All plants distributed finished water that was essentially free of indicator bacteria as judged by analysis of 1 liter for total coliforms, fecal coliforms, fecal streptococci, coagulase-positive staphylococci, and *Pseudomonas aeruginosa*. The total plate counts at 20 and 35°C were also evaluated as a measure of the total microbial population and were usually very low. Viruses were detected in 7% (11 of 155) of the finished water samples (1,000 liters) at an average density of 0.0006 MPNCU/liter the highest virus density measured being 0.02 MPNCU/liter. The average cumulative virus reduction was 95.15% after sedimentation and 99.97% after filtration and did not significantly decrease after ozonation or final chlorination. The viruses isolated from treated waters were all enteroviruses: poliovirus types 1, 2, and 3, coxsackievirus types B3, B4, and B5, echovirus type 7, and untyped picornaviruses.

The isolation of viruses from drinking water was a rare occurrence until a few years ago, when sensitive methods were developed for the detection of low numbers of enteric viruses in large volumes of water. These methods have been reviewed recently by Gerba and Goyal (5), and we have improved them in our laboratory and attained a good degree of success in the isolation of viruses from various types of water. In a first study of a filtration plant, we found viruses in most samples of finished water at levels from 0.001 to 0.1 per liter (13). Further sampling at this plant and two others during a 2-year period did not reveal any viruses in the finished water (19), but this collaborative study involved the use of BSC-1 cells which we later found were very inefficient for the isolation of enteric viruses from environmental samples. Refinement of our methods now enables us to detect enteric viruses at a mathematical level of detection of 0.003 most probable number of cytopathogenic units (MPNCU) per liter. We used these methods in a collaborative effort with the Ministry of Environment of Quebec to evaluate the removal of viruses during unit processes at seven water purification plants. The present report summarizes the final results and conclusions of this study, some of which are different from the preliminary results published earlier (16).

MATERIALS AND METHODS

Water treatment plants. Seven water treatment plants were selected in the Montreal area on the basis of source water quality, type of treatment(s), and age of the plant (Table 1). Treated water from each plant was sampled twice monthly for 12 months. Raw, postchlorination, and postsedimentation (sample volume, 100 liters) and postfiltration, postozonation, and finished-water (1,000 liters) samples were analyzed.

Virus concentration procedure. The virus concentration procedure is essentially the adsorption-elution procedure described previously (13, 16, 19). The virus concentration apparatus is composed of two filter holders containing the virus-adsorbing filters, an electric pump, and a proportional injector system. A different apparatus was used for each type of water and was used only for that purpose. Each system was rinsed with chlorinated water after use, and the filter holders were autoclaved with filters in place. The virus-adsorbing filters were electronegatively charged Diamond filter tubes (1-μm pore size) and Duo-Fine filters (0.25 μm pore size), both as 25.4-cm cartridges (Filterite Corporation, Timonium, Md.). The water to be sampled was filtered through the filters at 10 to 20 liters/min after conditioning to pH 3.5 (with 1,2-*N*-hydrochloric acid) and to 1 mM aluminum chloride. Each concentrator was equipped with an in-line pH probe permitting continuous pH monitoring. Chlorinated water was also dechlorinated with sodium thiosulfate at a final concentration of 5 mg/liter. After filtration of the required volume of water, as measured with a water meter, excess water was blown out, and the filters were brought back to the laboratory within 3 h.

To minimize the risk of contamination between samples, they were always processed from least contaminated (tap water) to most contaminated (raw water). Filters were eluted with 2 liters of autoclaved 0.5% beef extract (Difco Laboratories, Detroit, Mich.), pH 9.75, containing 0.1% Antifoam B (Dow Chemicals). Reconcentration to 25 ml was achieved by

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TABLE 1. Characteristics of the water treatment plants

Plant	Capacity (10^4 m^3/day)	Raw water quality[a]	Treatment process				
			Prechlorination	Coagulation and sedimentation	Filtration	Ozonation	Postchlorination
1	2.5	+++	+	+	+	+	+
2	4.5	++++	+	+	+	+	+
3	2.2	++++	Seasonal	+	+	+	+
4	0.9	++++	+	+	+	−	+[b]
5	5.5	++	+	+	+	−	+
6	100	+	−	−	+	−	+
7	NA[c]	+/−	−	−	−	−	+

[a] Raw water quality ranged from light (+/−) to heavy (++++) pollution.
[b] Chlorine dioxide used.
[c] NA, Not available.

organic flocculation at pH 3.5 in the presence of 1 mM ferric chloride (15). The pellet obtained after centrifugation at 3,200 × *g* was dissolved in 10 ml of a filter-sterilized solution containing 0.5 M glycine buffer, pH 9.0, 10% fetal bovine serum, 500 U of penicillin, 500 μg of streptomycin, and single-strength minimal essential medium with Earle salts (GIBCO Diagnostics, Madison, Wis.). All concentrated samples were completed to 25 ml with the same solution and treated with Freon 113 and antibiotics to reduce contamination and cell toxicity (14). Concentrated samples were kept at −70°C until analysis.

Virus isolation and cell cultures. Treated-water concentrates were inoculated undiluted into 10 25-cm^2 flasks (1 ml per flask) of Buffalo green monkey (BGM) cells; some were also assayed on Vero cells. Raw-water concentrates were inoculated undiluted and diluted 1:5, 1:25, and 1:125 in tissue culture medium in five flasks each. If all flasks at the highest dilution showed cytopathic effect, the concentrate was reinoculated after further dilution. All samples were submitted to two blind passages on the same cell line, and cells were maintained for 11 to 14 days at each passage, with observation every 3 to 4 days. Cultures showing maximal cytopathic effect were frozen immediately at −20°C, and the remaining cultures were frozen at the end of the incubation period.

BGM cells were obtained from Flow Laboratories, Inc., McLean, Va., and used at passages 75 to 90. Vero cells were obtained from the American Type Culture Collection, Bethesda, Md., and were used at passages 145 to 157. All cell cultures were maintained and passaged in minimal essential medium with Earle salts and maintained at 37°C.

Virus enumeration and identification. The number of viruses in the concentrates was estimated with a most-probable-number approach similar to that described by Koch (7) and expressed as MPNCU per liter of water. Calculations were performed on a Hewlett-Packard HP-3000 minicomputer.

Viruses detected in treated waters were identified by serum neutralization procedures with pooled specific antisera as described by Melnick et al. (9). Viruses not identified by these pooled sera were submitted to an immune electron microscopy procedure (2) to confirm that they were picornaviruses. Reoviruses were detected by an immunoassay similar to the one described previously for polioviruses (17). Because of the cost involved in identifying the very large number of viruses present in raw water samples, these viruses were not identified further.

Bacteriological analysis. Except for the standard plate count (total plate count), all bacteriological analyses were performed by membrane filtration methods on 1 liter of water if the turbidity permitted the filtration of such a volume. The standard plate count was performed at 20 and 35°C on 1 ml of water (1). The total coliform count was evaluated on endo-LES agar, fecal coliforms on m-FC agar, and fecal streptococci on m-enterococcus agar as recommended by the Ministry of Environment of Quebec (11).

The presence of *Pseudomonas aeruginosa* was evaluated at 41.5°C for 24 h on m-PA agar as recommended (1) except for the omission of sulfopyridine and actidione in the agar (3), and that of *Staphylococcus aureus* was evaluated at 35°C for 24 h on Baird-Parker agar containing 0.05 g of sulfamethazine (22) and 1.5 g of actidione (20) per liter. All shiny black colonies that were round and convex with a clear halo and a clear zone in the agar were considered *S. aureus* colonies. The identity of each colony was confirmed by Gram stain, catalase, DNase, thermonuclease, coagulase, and mannitol fermentation tests.

Physicochemical analysis. The following parameters were evaluated for all water samples: total and free residual chlorine concentration, ozone, turbidity, color, conductivity, alkalinity, and concentrations of aluminum, calcium, magnesium, manganese, silica, nitrate, nitrite, chlorides, fluorides, sulfates, total organic and inorganic carbon, total and dissolved solids, and ammonium nitrogen (1).

Except for chlorine and ozone measurements, all analyses were performed by the Laboratoire de Montréal (Ministère de l'Environnement). Chlorine and ozone measurements were performed on site with Hach Chemicals Co. test kits.

Statistical analysis. Coefficients of correlation (*R*) between the measured parameters were calculated with the Pearson and the Spearman tests (23).

RESULTS

Plant 1. Plant 1 draws raw water from a moderately polluted river receiving human, animal, and industrial wastes. Water samples could be obtained after 5 min of chlorination. The total heterotrophic aerobic population (standard plate count) was reduced by 1 $\log_{10}$ after these 5 min of contact in the presence of an average free chlorine residual concentration of 0.3 mg/liter (Table 2). Similar reductions were observed for total coliforms and fecal coliforms; fecal streptococci and *S. aureus* were completely eliminated, but *P. aeruginosa* remained relatively unaffected. Postsedimentation samples obtained after flocculation and sedimentation in the presence of residual chlorine were free of fecal coliforms, and the total coliform population was reduced by 90%. *P. aeruginosa* organisms were also considerably reduced, and only 1 of 15 postsedimentation water samples still contained this bacterium, which was eliminated by the filtration.

TABLE 2. Bacteriological and physicochemical data,[a] plant 1

Water sample	Standard plate count (log_{10} CFU/liter)		Organisms (log_{10} CFU/liter)					Chlorine (mg/liter)		pH	Turbidity (NTU)[b]	Color (TCU)[c]
	20°C	35°C	Total coliforms	Fecal coliforms	Streptococci	*S. aureus*	*P. aeruginosa*	Residual	Total			
Raw	7.2 (6.2–7.8)	7.0 (5.9–7.8)	4.6 (3.5–5.3)	3.8 (3.0–4.2)	2.9 (BD[d]–3.3)	0.6 (BD–1.8)	2.0 (BD–2.8)	NA[e]	NA	7.5 (7.1–8.0)	24.7 (6.0–80)	66.6 (30–200)
Chlorinated	6.2 (3.3–7.1)	6.3 (5.3–6.9)	3.3 (BD–4.3)	2.4 (BD–3.5)	BD	BD	2.0 (BD–2.8)	0.3 (0.2–0.8)	0.6 (0.3–1.1)	6.7 (6.3–7.2)	50.4 (0.8–100)	16.8 (3.0–80)
Sedimented	4.9 (3.9–5.8)	5.4 (4.3–6.3)	1.2 (BD–2.3)	BD	BD	BD	0.1 (BD–1.3)	0.3 (0.2–0.8)	0.5 (0.3–0.9)	6.9 (6.4–7.2)	6.8 (1.1–36)	9.4 (2.0–22)
Filtered	3.9 (BD–4.8)	4.7 (3.3–5.7)	0.5 (BD–1.5)	BD	BD	BD	BD	0.2 (BD–0.4)	0.4 (0.1–0.8)	6.8 (6.3–7.2)	0.8 (0.1–4.0)	3.8(BD–6.0)
Finished	ND[f]	ND	ND	ND	ND	ND	ND	0.3 (BD–0.6)	0.5 (0.1–1.3)	7.2 (7.0–7.5)	0.8 (0.1–2.0)	2.6(BD–6.0)

[a] All data are means, with the range shown in parentheses.
[b] NTU, Nephelometric turbidity units.
[c] TCU, True color units.
[d] BD, Below detection limits.
[e] NA, Not applicable.
[f] ND, Not determined.

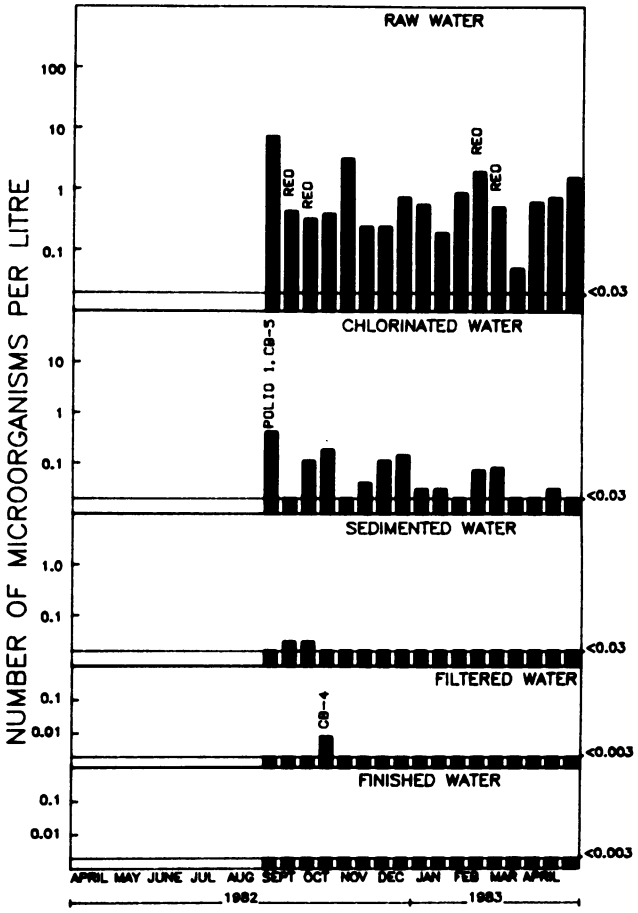


FIG. 1. Virus elimination at plant 1. REO, Reovirus; POLIO, poliovirus; C, coxsackievirus.

In raw water samples the mean virus density was 1.15 MPNCU/liter, and all samples contained viruses (Fig. 1). After a short prechlorination treatment, the virus density was 0.072 MPNCU/liter, and 11 of 17 samples contained viruses. Poliovirus type 1 and coxsackievirus B5 were detected in one of these 17 samples. Of 17 postsedimentation water samples, 2 contained viruses, with a mean virus density of 0.004 MPNCU/liter. Filtration further reduced the virus density to 0.005 MPNCU/liter, with only one postfiltration water sample containing coxsackievirus B4. None of the finished water samples contained virus; however, because of a possible cross-connection with a hot water system, these results were not considered further.

Plant 2. Plant 2 treats water from a river heavily polluted by human wastes. In postsedimentation water samples the standard plate count was reduced by 3 log_{10}, and indicator bacteria were absent from most samples except for total coliforms, but their concentration was already reduced by more than 4 log_{10} (Table 3). The number of *P. aeruginosa* organisms was reduced, but they were still present in most samples. Filtration removed remaining indicator microorganisms and leaved only traces of the total coliform population. The turbidity, pH, color, and conductivity of the finished water were within permissible limits.

The mean virus density in raw water samples was higher than at plant 1, 2.136 MPNCU/liter, and 4 of 25 postsedimentation water samples contained viruses (coxsackievirus B5,

TABLE 3. Bacteriological and physicochemical data, plant 2[a]

Water sample	Standard plate count (log_{10} CFU/liter)		Organisms (log_{10} CFU/liter)					Chlorine (mg/liter)		Ozone (mg/liter)	pH	Turbidity (NTU)[b]	Color (TCU)[c]
	20°C	35°C	Total coliforms	Fecal coliforms	Streptococci	*S. aureus*	*P. aeruginosa*	Residual	Total				
Raw	7.6 (5.5–8.9)	7.8 (5.9–9.1)	5.3 (4.3–6.2)	4.3 (3.0–5.1)	3.4 (2.5–4.2)	1.3 (BD–2.0)	3.3 (BD–4.5)	NA	NA	NA	7.2 (6.4–7.8)	16.4 (2.7–85)	37.3 (2.0–52)
Sedimented	4.8 (BD–5.8)	5.3 (3.3–6.2)	0.8 (BD–2.0)	BD	−0.4 (BD–1.0)	0.0 (BD–1.3)	1.9 (BD–2.9)	0.1 (0.0–0.7)	0.2 (0.0–1.0)	NA	6.7 (6.1–7.2)	2.7 (0.5–15.4)	10.4 (BD–23.0)
Filtered	3.0 (BD–3.8)	3.3 (BD–3.8)	−0.5 (BD–0.8)	BD	BD	BD	−1.4 (BD–0.0)	0.1 (0.0–0.3)	0.2 (0.0–0.5)	NA	6.6 (6.0–7.2)	0.6 (0.1–1.9)	5.7 (0–10)
Ozonated	3.6 (BD–4.7)	4.8 (BD–5.8)	1.1 (BD–2.5)	BD	−1.4 (BD–0)	BD	−0.4 (BD–0.9)	NA	NA	0.1 (0.0–0.2)	6.6 (6.0–7.1)	0.8 (0.1–2.0)	4.7 (BD–8.0)
Finished	3.2 (BD–4.3)	4.0 (BD–5.2)	0.2 (BD–1.6)	BD	BD	BD	−1.4 (BD–0.0)	0.9 (0.0–1.4)	1.1 (0.1–1.8)	NA	6.7 (5.4–7.4)	0.5 (0.1–1.7)	2.9 (BD–6.0)

[a] All data are means, with the range shown in parentheses. See the footnotes to Table 2 for other explanations.

TABLE 4. Bacteriological and physicochemical data, plant 3[a]

Water sample	Standard plate count (log_{10} CFU/liter)		Organisms (log_{10} CFU/liter)					Chlorine (mg/liter)		Ozone (mg/liter)	pH	Turbidity (NTU)[b]	Color (TCU)[c]
	20°C	35°C	Total coliforms	Fecal coliforms	Streptococci	*S. aureus*	*P. aeruginosa*	Residual	Total				
Raw	7.3 (6.0–7.7)	7.4 (5.9–7.7)	6.0 (4.9–6.6)	4.2 (BD–4.6)	4.2 (BD–5.3)	1.1 (BD–2.0)	4.0 (BD–4.8)	NA	NA	NA	7.3 (6.2–7.7)	22.3 (2.9–100)	41.6 (14–74)
Sedimented	6.1 (BD–7.0)	6.1 (4.2–7.1)	4.2 (BD–5.3)	2.5 (BD–3.8)	2.1 (BD–3.3)	BD	3.1 (BD–4.4)	0.1 (BD–0.3)	0.1 (BD–0.4)	NA	6.9 (6.3–7.5)	0.9 (BD–3.5)	4.8 (2.0–11.0)
Filtered	6.5 (BD–6.4)	5.6 (4.3–6.1)	2.8 (BD–3.8)	1.0 (BD–1.9)	0.8 (BD–0.6)	BD	0.5 (BD–0.7)	0.1 (BD–0.9)	0.1 (BD–1.0)	NA	6.8 (6.5–7.4)	0.3 (BD–0.8)	4.7 (1.0–34.0)
Ozonated	3.1 (BD–4.0)	3.4 (BD–4.0)	−1.0 (BD–0.0)	BD	BD	BD	BD	NA	NA	0.8 (BD–1.5)	6.6 (6.1–7.3)	0.4 (BD–1.6)	2.9 (BD–20.0)
Finished	2.6 (BD–3.7)	3.4 (BD–4.5)	0.2 (BD–1.6)	BD	BD	BD	BD	0.8 (0.4–1.4)	1.0 (BD–2.3)	NA	7.4 (6.8–8.2)	0.4 (BD–0.9)	4.1 (1.0–16.0)

[a] All data are means, with the range shown in parentheses. See the footnotes to Table 2 for other explanations.

TABLE 5. Bacteriological and physicochemical data, plant 4[a]

Water sample	Standard plate count (log_{10} CFU/liter)		Organisms (log_{10} CFU/liter)					Chlorine (mg/liter)		pH	Turbidity (NTU)[b]	Color (TCU)[c]
	20°C	35°C	Total coliforms	Fecal coliforms	Streptococci	*S. aureus*	*P. aeruginosa*	Residual	Total			
Raw	7.1 (6.4–7.6)	7.5 (6.3–8.7)	5.5 (4.1–6.3)	4.3 (BD–4.6)	3.2 (BD–3.5)	1.2 (BD–2.0)	3.7 (2.0–4.8)	NA	NA	7.3 (5.8–7.8)	13.5 (3.0–40.0)	46.6 (2.0–130)
Sedimented	5.1 (BD–6.4)	5.1 (BD–6.2)	1.2 (BD–2.6)	−1.1 (BD–0.3)	−0.8 (BD–0.3)	1.1 (BD–2.5)	1.8 (BD–2.7)	0.1 (BD–0.4)	0.3 (BD–0.9)	6.6 (5.8–7.1)	2.9 (1.0–7.0)	9.9 (3.0–22.0)
Filtered	6.0 (BD–7.4)	5.5 (BD–6.6)	0.5 (BD–1.8)	BD	BD	BD	−0.6 (BD–0.8)	0.1 (BD–0.4)	0.3 (BD–0.6)	6.5 (6.1–7.0)	0.7 (0.1–4.0)	4.5 (BD–18.0)
Finished	3.2 (BD–4.3)	3.7 (BD–4.4)	−1.4 (BD–0.0)	BD	BD	BD	BD	0.5 (0.2–0.8)	0.6 (0.4–1.0)	7.5 (7.0–8.5)	0.7 (0.2–3.0)	4.8 (BD–10.0)

[a] All data are means, with the range shown in parentheses. See the footnotes to Table 2 for other explanations.

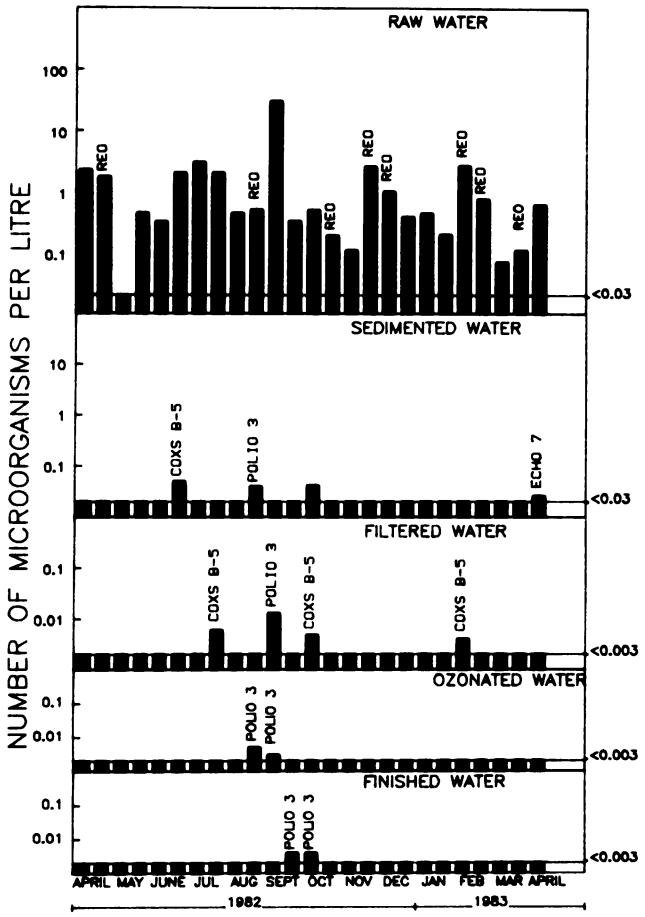


FIG. 2. Virus elimination at plant 2. REO, Reovirus; COXS, coxsackievirus; POLIO, poliovirus; ECHO, echovirus.

poliovirus type 3, and echovirus type 7) at a mean density of 0.0067 MPNCU/liter (Fig. 2). Filtration reduced the average virus density to 0.0011 MPNCU/liter, with 4 of 25 postfiltration samples containing coxsackievirus type B5 and two postozonation and finished water samples containing poliovirus type 3 at an average density of 0.0003 MPNCU/liter.

Plant 3. Plant 3 treats water from a low-flow-rate river heavily polluted by untreated human and animal wastes. The animal waste pollution is due to pig farm effluents and was reflected by the higher ratio of fecal streptococci to coliforms in the raw water. Raw water was prechlorinated only during the warmer months when the pollution load is greater, and the averaged data (Table 4) do not reflect this practice. The higher levels of microorganisms in postsedimentation and postfiltration samples reflect the breakthrough of bacteria when water was not prechlorinated. The seasonal impact of prechlorination is shown in Fig. 3B and C. A breakthrough of bacteria in the absence of prechlorination was evident during the spring in postsedimentation and postfiltration samples. Postfiltration water samples contained bacteria which were eliminated by ozonation. Two finished water samples contained 1 and 36 total coliforms per liter. The turbidity, pH, color, and residual chlorine concentration were all within permissible limits.

The mean virus density in the raw water was 3.588 MPNCU/liter, and all samples contained viruses (Fig. 3A). Postsedimentation, 5 of 25 samples contained viruses at a mean density of 0.019 MPNCU/l. Poliovirus types 2 and 3, one coxsackievirus type B4, and an untyped picornavirus were identified. Postfiltration, the virus density was reduced to 0.0013 MPNCU/liter, and viruses were isolated from six samples, including poliovirus types 2 and 3 and an untyped picornavirus. Postozonation water samples contained viruses on two occasions at a virus density of 0.0004 MPNCU/liter. Of 25 finished water samples, 4 contained viruses for a mean density of 0.0007 MPNCU/liter, and all isolates were poliovirus type 3.

Plant 4. Plant 4 draws its raw water from a heavily polluted stream which receives untreated domestic and industrial wastes. After chlorination, raw water is treated with alum and activated silica. This treatment reduces dramatically the bacterial load, and although several samples were positive, the average concentration was less than 20 CFU/liter for most of the indicator bacteria measured (Table 5). *P. aeruginosa* was relatively unaffected by the treatment. Filtration removed the remaining bacteria, and only one postfiltration sample was positive for total coliforms and one for *P. aeruginosa*. One sample of finished water contained 1 total coliform per liter. The turbidity, pH, color, and conductivity values were all within permissible limits. This plant used chlorine dioxide, and a free chlorine residual was maintained in the finished water.

All samples of raw water contained viruses at a mean density of 13.3 MPNCU/liter, with two samples collected in June and July containing 128 MPNCU/liter (Fig. 4). Reoviruses were frequently found in raw water samples. Of 27 postsedimentation samples, 7 contained viruses at an average density of 0.03 MPNCU/liter. Coxsackievirus types B3, B4, and B5 and poliovirus type 3 were isolated from these samples. Postfiltration water samples contained the same virus types except for coxsackievirus B3, and the mean virus density was 0.002 MPNCU/liter, with 6 of 27 samples positive for viruses. Only 1 of 27 finished water samples contained viruses, at a density of 0.02 MPNCU/liter.

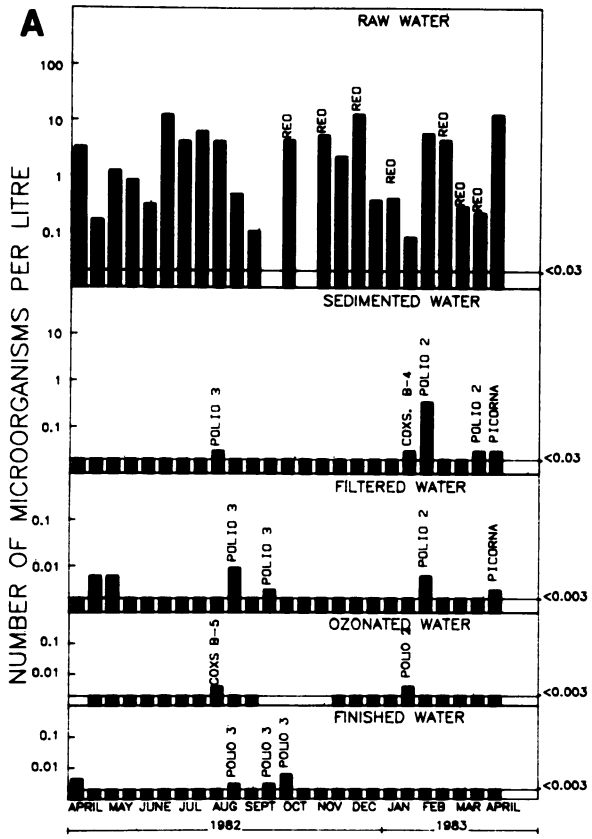
Plant 5. Plant 5 draws its raw water from a large river moderately polluted by domestic and industrial wastes. Water treatments are similar to those at plant 4 and resulted in similar elimination of bacterial contaminants. The physicochemical test values of the finished water samples were within permissible values, except for one sample which did not contain free residual chlorine (Table 6).

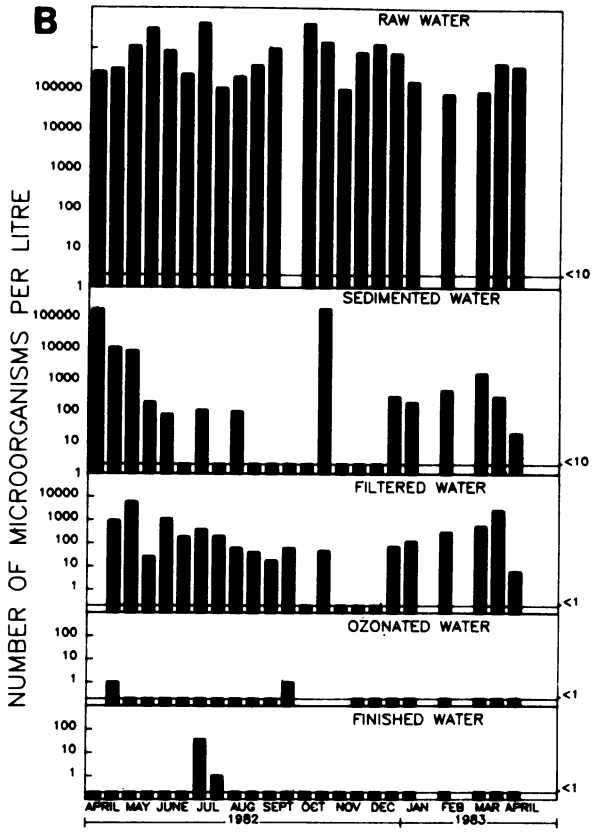
Raw water samples contained a mean virus density of 0.52 MPNCU/liter (Fig. 5). Of 25 postsedimentation samples, 5 contained viruses at an average density of 0.016 MPNCU/liter. Poliovirus type 3, coxsackievirus B5, and an untyped picornavirus were identified. Postfiltration water samples were free of viruses, but 2 of 25 finished water samples contained poliovirus type 3 and coxsackievirus B5.

Plant 6. Plant 6 draws its raw water from the same stream as plant 5 but at a point where the water quality is better. Weater treatment is minimal, using only filtration and chlorination. This was the only plant where finished water samples contained staphylococci on one occasion (Table 7). One total coliform per liter was found on one occasion. The physicochemical test values for the finished water were within permissible limits.

Raw water was relatively free of viruses, with only 5 of 25 samples containing viruses at a mean density of 0.033 MPNCU/liter (Fig. 6). Three finished water samples contained viruses: two poliovirus type 3 and a coxsackievirus B5.

Plant 7. Plant 7 is a pumping and chlorination station which draws raw water from a shallow bay. Turbidity and





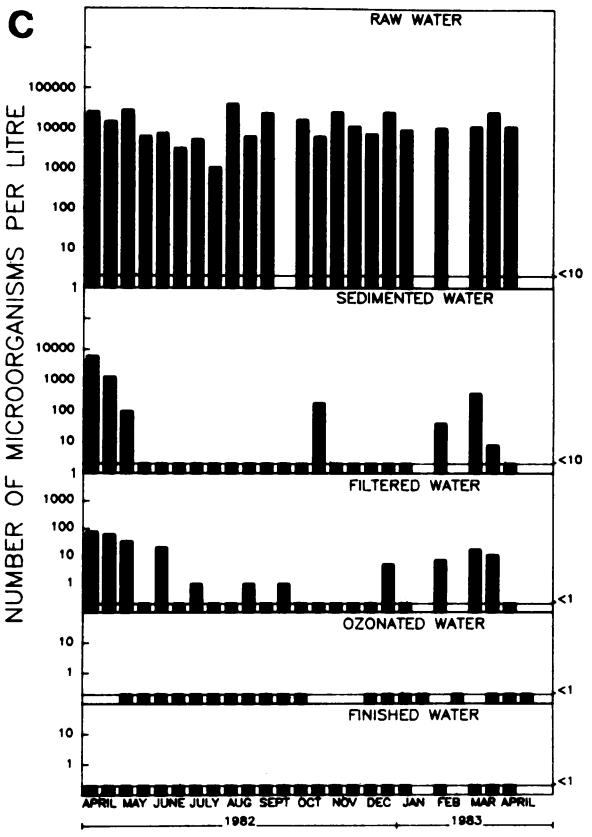


FIG. 3. Elimination of viruses (A), total coliform bacteria (B), and fecal coliform bacteria (C) at plant 3. Abbreviations: see Fig. 2 legend.

pollution levels are variable according to prevailing atmospheric conditions. Several times a year residents are advised to boil their water because of a high-risk situation, e.g., high turbidity. Some samples were taken when the water was considered unsafe to drink. Chlorine demand is very high, but a free residual chlorine level is usually maintained in the distribution system. Turbidity values often exceed permissible limits and are the main reason for considering this water unsafe for drinking. The distributed water was free of any of the indicator bacteria measured, and the total bacterial population was low (Table 8). Only 1 of the 11 raw water samples and none of the finished water samples contained virus.

Statistical analysis. To detect a possible correlation between the presence of viruses and any of the mirobiological parameters measured, we used the Spearman and the Pearson correlation tests (23) (Table 9). The presence of viruses in raw water was weakly correlated with some of the parameters measured: the standard plate count at 20 or 35°C and total and fecal coliforms being in the $r = 0.5$ to 0.7 range. In treated samples, none of the measured parameters correlated with the presence of viruses.

DISCUSSION

This study was undertaken to evaluate the efficiency of several water treatment plants in eliminating indicator bacteria and viruses under various pollution loads of their raw water. All plants delivered water which was bacteriologi-

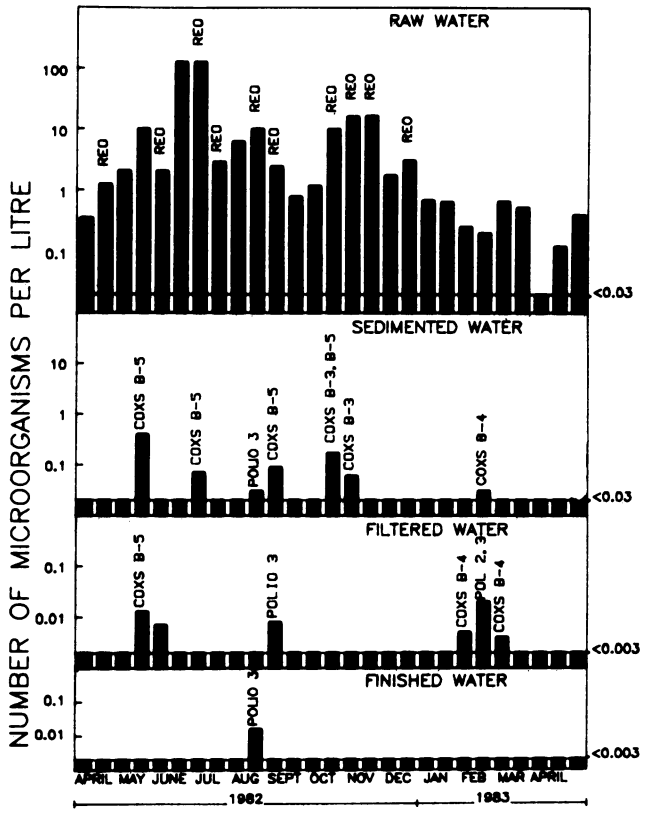


FIG. 4. Virus elimination at plant 4. Abbreviations: see Fig. 2 legend.

cally safe as determined by analysis of 1 liter. Indicator bacteria were essentially eliminated before filtration, and filtration efficiently removed most of the bacteria that had survived the earlier treatments. Ozonation and chlorination eliminated the residual bacteria. We have little data (17 samples) to evaluate the effect of prechlorination of the raw water on virus density, but the mean virus density after prechlorination at plant 1 indicates that a reduction of over 95% was achieved after about 5 min at a free residual chlorine level of 0.5 to 1 mg/liter (Tables 10 and 11).

The prechlorination-coagulation-sedimentation process appears to be the most efficient step in reducing the number of microorganisms in the water. Even in the presence of more than several million coliforms per liter, the finished water was always free of any of the indicator bacteria, and the residual fraction in postsedimentation water samples was minimal. Information of this type is obtained daily, as most water treatment plants continuously produce water that is free of detectable indicator bacteria. Furthermore, breakthroughs are relatively rare and can usually be traced to a faulty treatment and not to resistant microorganisms.

Our study has shown that the degree of virus elimination was not as great as that observed for other indicators. Whereas the total coliform population was reduced from several million to less than 1 per liter (6 $\log_{10}$ reduction), the virus population was reduced, at maximum efficiency, from 125 to less than 0.003 MPNCU/liter (4 to 5 $\log_{10}$ reduction). The overall observed virus reduction (about 4 $\log_{10}$) (Table 11) was certainly sufficient to reduce the health risk to a minimal level: the presence of one virus infectious unit in several liters of finished water (found in fewer than 20 of 1,000 liters studied), although unassessed experimentally, is so far below the observed infectious doses for most enteric viruses (12) that the risk of infection appears insignificant (6). The observed virus reduction was, however, far from the 12 $\log_{10}$ reduction expected for direct reclamation of drinking water from wastewater (25). Stetler et al. (24) have also shown that viruses are present in large numbers in postsedimentation and postfiltration water samples and that 50% of the postfiltration samples and more finished water samples (380 liters) contained viruses. In the present study viruses were found in most of the treated water samples, but it is of interest that the only plant distributing virus-free water was treating almost virus-free raw water. Furthermore, the presence of viruses in finished water was in correlation with their presence in raw water, showing the importance of sewage treatment in reducing the virus load in source water.

Even if we were able to detect about 3 MPNCU/1,000 liters, we could not demonstrate a correlation between the indicator bacteria and viruses measured. Because all finished water samples were essentially free of indicator bacteria (coliforms, staphylococci, streptococci, and pseudomonads), we considered these waters safe to drink even though they contained some viruses.

It is evident that the viruses isolated from the treated waters are among the most resistant of the enteric viruses. Although raw water samples contained large numbers of reoviruses, these viruses were not found in treated waters. The presence of enteroviruses in treated waters is certainly an indication of their resistance to inactivation; Liu et al. (8)

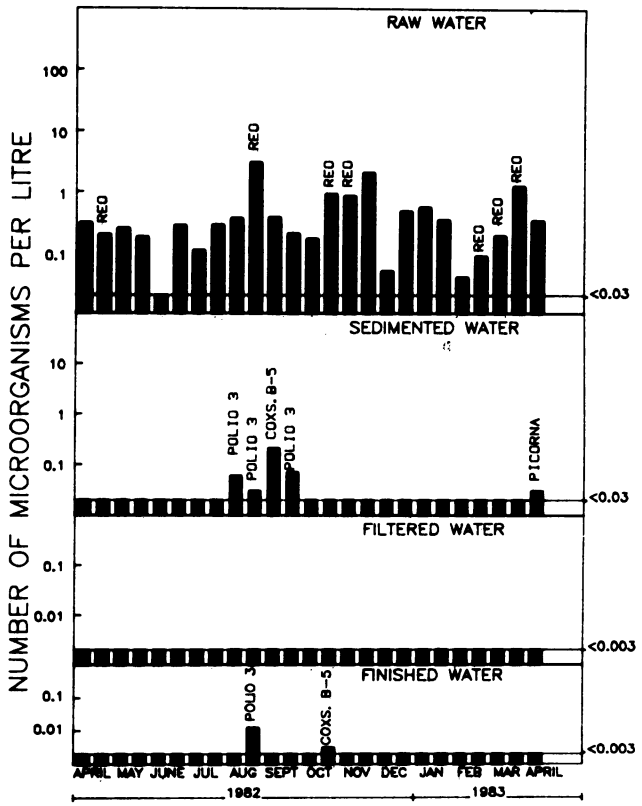


FIG. 5. Virus elimination at plant 5. Abbreviations: see Fig. 2 legend.

TABLE 6. Bacteriological and physicochemical data, plant 5[a]

Water sample	Standard plate count (log_{10} CFU/liter)		Total coliforms	Fecal coliforms	Streptococci	*S. aureus*	*P. aeruginosa*	Chlorine (mg/liter)		pH	Turbidity (NTU)[b]	Color (TCU)[c]
	20°C	35°C						Residual	Total			
Raw	6.7 (6.1–7.3)	6.7 (6.3–7.3)	5.0 (4.4–5.4)	4.0 (3.2–4.4)	3.1 (1.9–3.6)	1.2 (BD–2.0)	4.1 (BD–5.3)	NA	NA	7.6 (7.0–8.3)	8.7 (1.5–40.0)	28.4 (4.0–60.0)
Sedimented	6.0 (3.0–7.0)	5.8 (3.7–6.7)	1.1 (BD–1.9)	−0.2 (BD–0.9)	−0.4 (BD–1.8)	BD	2.3 (BD–3.4)	0.1 (BD–0.3)	0.2 (BD–0.5)	7.1 (6.4–7.9)	2.3 (0.6–4.5)	7.8 (BD–16.0)
Filtered	5.4 (BD–6.8)	5.6 (BD–6.9)	0.4 (BD–1.5)	BD	−0.2 (BD–1.0)	BD	−0.5 (BD–0.9)	BD	0.1 (BD–0.5)	6.9 (6.2–7.6)	0.5 (0.1–1.5)	3.5 (BD–8.0)
Finished	3.0 (BD–3.9)	3.1 (BD–3.8)	BD	BD	BD	BD	BD	0.3 (BD–0.5)	0.4 (0.2–0.6)	7.4 (6.8–8.1)	0.4 (0.1–1.8)	4.1 (2.0–10.0)

[a] All data are means, with the range shown in parentheses. See the footnotes to Table 2 for other explanations.

TABLE 7. Bacteriological and physicochemical data, plant 6[a]

Water sample	Standard plate count (log_{10} CFU/liter)		Organisms (log_{10} CFU/liter)					Chlorine (mg/liter)		pH	Turbidity (NTU)[b]	Color (TCU)[c]
	20°C	35°C	Total coliforms	Fecal coliforms	Streptococci	*S. aureus*	*P. aeruginosa*	Residual	Total			
Raw	6.1 (5.1–6.5)	6.0 (5.1–6.6)	3.4 (BD–4.1)	2.7 (BD–3.2)	2.4 (BD–2.8)	BD	3.9 (BD–5.0)	NA	NA	7.9 (7.3–8.4)	2.1 (0.9–5.3)	8.2 (1.0–23.0)
Finished	3.8 (BD–4.6)	4.0 (BD–4.9)	−1.4 (BD–0.0)	BD	BD	−0.7 (BD–0.7)	BD	0.5 (0.4–0.8)	0.6 (0.4–0.9)	7.8 (7.4–8.2)	0.7 (0.1–2.0)	3.8 (BD–25.0)

[a] All data are means, with the range shown in parentheses. See the footnotes to Table 2 for other explanations.

TABLE 8. Bacteriological and physicochemical data, plant 7[a]

Water sample	Standard plate count (log_{10} CFU/liter)		Organisms (log_{10} CFU/liter)					Chlorine (mg/liter)		pH	Turbidity (NTU)[b]	Color (TCU)[c]
	20°C	35°C	Total coliforms	Fecal coliforms	Streptococci	*S. aureus*	*P. aeruginosa*	Residual	Total			
Raw	6.4 (3.9–7.2)	6.2 (4.3–7.0)	3.5 (BD–4.1)	2.0 (BD–3.0)	2.3 (BD–3.3)	BD	0.9 (BD–1.9)	NA	NA	7.8 (7.4–8.4)	3.4 (0.6–22.0)	7.0 (4.0–14.0)
Finished	3.8 (BD–4.4)	4.0 (BD–4.5)	BD	BD	BD	BD	BD	0.4 (BD–0.9)	0.5 (0.1–1.0)	7.9 (7.5–8.5)	3.5 (0.6–24.0)	5.7 (2.0–14.0)

[a] All data are means, with the range shown in parentheses. See the footnotes to Table 2 for other explanations.

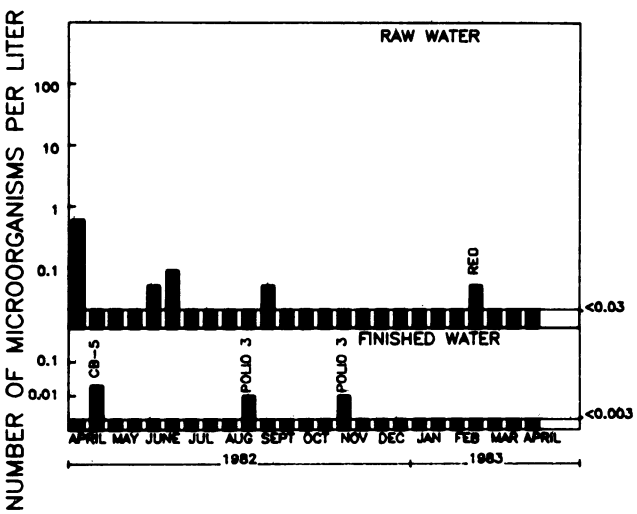


FIG. 6. Virus elimination at plant 6. Abbreviations: see Fig. 1 legend.

have shown that in river water reoviruses were reduced by 4 $\log_{10}$ in the presence of free chlorine in less than 5 min, whereas poliovirus types 2 and 3 and coxsackievirus B3 and B5 required 30 to 40 min for similar reduction.

The presence of viruses in treated waters and especially finished waters remains to be explained. Most of the viruses isolated in this study were tested to determine their resistance to 0.5 mg of free chlorine per liter over a period of 18 h (18). Whereas the poliovirus isolates were highly sensitive to chlorine, coxsackievirus B4 and B5 isolates were generally more resistant to the same chlorine concentration. After 16 h of contact with chlorine, the residual surviving fraction was less than 0.001% for poliovirus but about 0.015% for coxsackievirus B4 and about 0.05% for coxsackievirus B5. Resistance could thus explain the presence of coxsackieviruses but not of polioviruses in finished water. The former may have been protected from disinfection by another mechanism, e.g., occlusion in protective matter.

The methodology we have used for recovering viruses from water is certainly one of the most efficient methods developed for that purpose. In a recent comparative evaluation of available methods, Melnick et al. (10) showed that the Viradel (virus adsorption-elution) method, using cartridge-type Filterite filters and organic flocculation for the reconcentration of eluates, was the most efficient. Furthermore, although many laboratories are still using the plaque-forming assay system for enumerating viruses in concentrated samples, we have used the liquid overlay method which permits multiple passages and the detection of slow-growing viruses and those not producing plaques. The detection of reoviruses in the raw water samples and of

TABLE 9. Correlation analysis[a] of virus density with bacteriological data

Water sample (no. of samples)	Standard plate count		Total coliforms	Fecal coliforms	Streptococci	*S. aureus*	*P. aeruginosa*
	20°C	35°C					
Raw (144)							
*R*1	0.56	0.62	0.68	0.59	0.45	0.19	0.41
*R*2	0.56	0.63	0.67	0.63	0.48	0.19	0.34
Chlorinated (15)							
*R*1	0.34	0.25	0.07	0.04	NA	NA	0.05
*R*2	0.24	0.39	0.03	0.15	NA	NA	0.04
Sedimented (112)							
*R*1	0.19	0.10	0.03	0.001	−0.08	−0.06	0.06
*R*2	0.17	0.22	0.08	−0.003	−0.004	−0.07	0.04
Filtered (111)							
*R*1	0.09	0.02	0.09	0.12	−0.06	NA	0.20
*R*2	0.14	0.06	0.10	0.18	−0.07	NA	0.23
Ozonated (42)							
*R*1	0.11	0.07	−0.06	NA	−0.04	NA	−0.05
*R*2	0.01	0.04	−0.09	NA	0.04	NA	0.06
Finished (145)							
*R*1	0.06	−0.02	−0.04	NA	NA	0.02	−0.02
*R*2	0.11	−0.05	−0.06	NA	NA	0.02	−0.03

[a] *R*1, Pearson test; *R*2, Spearman test; NA, not applicable because all data for one or both variables were negative.

TABLE 10. Elimination of viruses at seven water purification plants

Water sample	No. of samples positive for virus/no. tested (mean virus density [MPNCU/liter])						
	Group 1			Group 2		Group 3	
	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7
Raw	17/17 (1.150)	24/25 (2.136)	23/23 (3.588)	26/26 (13.31)	24/25 (0.52)	5/25 (0.03)	1/11 (0.004)
Chlorinated	11/17 (0.072)	NS[a]	NS	NS	NS	NA[b]	NA
Sedimented	2/17 (0.004)	4/25 (0.007)	5/25 (0.019)	7/27 (0.030)	5/25 (0.016)	NA	NA
Filtered	1/17 (0.0005)	4/25 (0.0011)	6/25 (0.0013)	6/27 (0.0021)	0/25 (0.0000)	NS	NA
Ozonated	NS	2/25 (0.0003)	2/20 (0.0004)	NA	NA	NA	NA
Finished	NS	2/25 (0.0003)	4/25 (0.0007)	1/27 (0.0007)	2/25 (0.0005)	3/25 (0.0014)	0/11 (0.0000)

[a] NS, Not sampled.
[b] NA, Not applicable.

TABLE 11. Elimination of viruses: group data

Group and water sample	No. positive samples/total (% positive)	Mean virus density (MPNCU/liter)	% of virus remaining
Group 1			
Raw	64/65 (99)	2.392	100
Chlorinated	11/17 (65)	0.072	3.01
Sedimented	11/67 (16)	0.010	0.42
Filtered	11/67 (16)	0.0010	0.042
Ozonated	4/45 (9)	0.0003	0.013
Finished	6/50 (12)	0.0005	0.020
Group 2			
Raw	50/51 (98)	6.93	100
Sedimented	12/52 (23)	0.023	0.33
Filtered	6/52 (12)	0.0011	0.016
Finished	3/52 (6)	0.0006	0.009
Group 3			
Plant 6			
Raw	5/25 (20)	0.03	100
Finished	3/25 (12)	0.0014	4
Plant 7			
Raw	1/11 (9)	0.004	100
Finished	0/11 (0)	0.000	0
All groups			
Raw	120/152 (79)	3.36	100
Chlorinated	11/17 (65)	0.072	2.1
Sedimented	23/119 (20)	0.016	0.47
Filtered	17/119 (14)	0.001	0.03
Ozonated	4/45 (9)	0.0003	0.009
Finished	12/138 (9)	0.0006	0.018

several of the viruses isolated after water treatment was possible only through the use of that methodology.

Conclusions. Until a few years ago the isolation of viruses from drinking water was a rare occurrence and was not reported in the literature because of the implications of such reports. It was, however, clear that several such isolations had taken place and were dismissed by government officials or simply discussed in the closed circles of environmental virologists. With the advent of more sophisticated methods for the detection of viruses in water, it was expected that the number of reports on the isolation of viruses from treated water would increase. This is the third time that viruses have been reported in drinking water in Canada, twice by workers in our laboratory and once by Sekla et al. (21), and we expect that several groups will report similar results throughout the world, as was recently done during a symposium on water disinfection in England (4).

Achieving a 12 $\log_{10}$ virus reduction and having a zero virus tolerance for finished waters are completely unrealistic goals. Virus standards for raw waters do appear to be more important and are needed, as are realistic finished water standards.

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LITERATURE CITED

1. **American Public Health Association.** 1980. Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, Inc., New York.
2. **Berthiaume, L., R. Alain, B. McLaughlin, P. Payment, and P. Trépanier.** 1981. Rapid detection of human viruses in faeces by a simple and routine immune electron microscopy technique. J. Gen. Virol. **55**:223–227.
3. **Brodsky, H. H., and W. B. Ciebin.** 1978. Improved medium for recovery and enumeration of *Pseudomonas aeruginosa* from water using membrane filters. Appl. Environ. Microbiol. **36**:36–42.
4. **Buttler, M., A. R. Medlen, and R. Morris.** 1982. Viruses and disinfection of water and wastewater. Proceedings of the International Symposium, University of Surrey, Guildford, 1 to 4 September 1982. University of Surrey Print Unit, Guildford, U.K.
5. **Gerba, C. P., and S. M. Goyal.** 1982. Methods in environmental microbiology. Marcel Dekker, Inc., New York.
6. **IAWPRC Study Group on Water Virology.** 1983. The health significance of viruses in water. Water Res. **17**:121–132.
7. **Koch, A. L.** 1982. Estimation of the most-probable-number with a programmable pocket calculator. Appl. Environ. Microbiol. **43**:488–490.
8. **Liu, O. C., H. R. Seraichekas, E. W. Akin, D. A. Brashear, E. L. Katz, and W. J. Hill.** 1971. Relative resistance of 20 human enteric viruses to free chlorine in Potomac water. Proceedings of the 13th Water Quality Conference, Virus and Water Quality: Occurrence and Control. University of Illinois, Urbana-Champaign, Ill.
9. **Melnick, J. L., V. Rennick, B. Hamphil, N. J. Schmidt, and H. H. Ho.** 1973. Lyophilised combination pools of enterovirus equine antisera: preparation and test procedures for the identification of field strains of 42 enteroviruses. Bull. W.H.O. **48**:263–268.
10. **Melnick, J. L., R. Safferman, V. C. Rao, S. Goyal, G. Berg, D. R. Dahling, B. A. Wright, E. Akin, R. Stetler, C. Sorber, B. Moore, M. D. Sobsey, R. Moore, A. A. Lewis, and F. M. Wellings.** 1984. Round robin investigation of methods for the recovery of poliovirus from drinking water. Appl. Environ. Microbiol. **47**:144–150.
11. **Ministère de l'Environnement du Québec.** 1981. Guide d'évaluation de la qualité bactériologique de l'eau potable. Editeur Officiel du Québec, Quebec, Canada.
12. **Minor, T. E., C. I. Allen, A. A. Tsiatis, D. B. Nelson, and D. J. D'Alessio.** 1981. Human infective dose for oral poliovirus type 1 vaccine in infants. J. Clin. Microbiol. **13**:388–389.
13. **Payment, P.** 1981. Isolation of viruses from drinking water at the Pont-Viau water treatment plant. Can. J. Microbiol. **27**:417–420.
14. **Payment, P., R. Ayache, and M. Trudel.** 1983. A survey of enteric viruses in domestic sewage. Can. J. Microbiol. **29**:111–119.
15. **Payment, P., S. Fortin, and M. Trudel.** 1984. Ferric chloride flocculation for nonflocculating beef extract preparations. Appl. Environ. Microbiol. **47**:591–592.
16. **Payment, P., R. Plante, and M. Trudel.** 1983. Elimination des bactéries et des virus lors de la purification des eaux de consommation. Sci. Tech. l'Eau **16**:251–254.
17. **Payment, P., C. Tremblay, and M. Trudel.** 1982. Rapid identification and serotyping of poliovirus isolates by an immunoassay. J. Virol. Methods **5**:301–308.
18. **Payment, P., M. Tremblay, and M. Trudel.** 1985. Relative resistance to chlorine of poliovirus and coxsackievirus isolates from environmental sources and drinking water. Appl. Environ. Microbiol. **49**:981–983.

19. **Payment, P., M. Trudel, S. A. Sattar, S. Springthorpe, T. P. Subrahmanyan, B. E. Gregory, A. H. Vajdic, P. Blaskovic, I. J. Guglielmi, and O. Kudrewko.** 1984. Virological examination of drinking water: a Canadian collaborative study. Can. J. Microbiol. **30**:105–112.
20. **Qureshi, A. A.** 1978. Microbiological characteristics of storm water run-offs at East York (Toronto) and Guelph separate storm sewers. Environment Canada and Ontario Ministry of the Environment, research report no. 87. Environment Canada, Ottawa, Ontario.
21. **Sekla, L., W. Stackiw, C. Kay, and L. Van Buckenhout.** 1980. Enteric viruses in renovated water in Manitoba. Can. J. Microbiol. **26**:518–523.
22. **Smith, B. A., and A. C. Baird-Parker.** 1964. The use of sulphamezathine for inhibiting *Proteus sp.* on Baird-Parker's isolation medium for *Staphylococcus aureus*. J. Appl. Bacteriol. **27**:78–82.
23. **Snedecor, G. W., and W. G. Cochran.** 1971. Statistical methods, p. 172–195. Iowa State University Press, Ames, Iowa.
24. **Stetler, R. E., R. L. Ward, and S. C. Waltrip.** 1983. Enteric virus and indicator bacteria levels in a water treatment system modified to reduce trihalomethane production. Appl. Environ. Microbiol. **47**:319–324.
25. **World Health Organization Scientific Group.** 1979. Human viruses in water, wastewater and soil. Technical report series 639. World Health Organization, Geneva.