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Viral Pollution of Surface Waters Due to Chlorinated Primary Effluents

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The role of chlorinated primary effluents in viral pollution of the Ottawa River (Ontario) was assessed by examining 282 field samples of wastewaters from two different sewage treatment plants over a 2-year period. The talc-Celite technique was used for sample concentration, and BS-C-1 cells were employed for virus detection. Viruses were detected in 80% (75/94) of raw sewage, 72% (68/94) of primary effluent, and 56% (53/94) of chlorinated effluent samples. Both raw sewage and primary effluent samples contained about 100 viral infective units (VIU) per 100 ml. Chlorination produced a 10- to 50-fold reduction in VIU and gave nearly 2.7 VIU/100 ml of chlorinated primary effluent. With a combined daily chlorinated primary effluent output of approximately 3.7×10^8 liters, these two plants were discharging 1.0×10^{10} VIU per day. Because the river has a mean annual flow of 8.0×10^{10} liters per day, these two sources alone produced a virus loading of 1.0 VIU/8 liters of the river water. This river also receives at least 9.0×10^7 liters of raw sewage per day and undetermined but substantial amounts of storm waters and agricultural wastes. It is used for recreation and acts as a source of potable water for some 6.0×10^5 people. In view of the potential of water for disease transmission, discharge of such wastes into the water environment needs to be minimized.

Although it is now well recognized (1, 19) that primary treatment (sedimentation) of sewage followed by chlorination of the effluent is relatively inefficient in the removal of inactivation of enteric viruses, many communities continue to rely on it for the treatment of their liquid wastes. Limited quantitative data are available on the contribution of such inadequately treated wastes to viral pollution of the water environment. The basic aim of this investigation was, therefore, to assess the virus loading of a river receiving chlorinated primary effluents from an urban center.

The city of Ottawa (latitude, 45°25′ N; longitude, 75°42′ W), Ontario, with its suburbs, represents a population of approximately 5.0×10^5. At the time of this investigation, more than 90% of the sewage (predominantly of domestic origin) generated here was being subjected to primary treatment and chlorination and discharged into the Ottawa River.

Because one of the two treatment plants studied here was relatively small, handling only 3.0×10^6 liters of sewage per day, we found it possible to collect from it temporally coordinated samples. The importance of such sampling in the study of waste treatment has been emphasized (2).

MATERIALS AND METHODS

Cells. BS-C-1 cells (12) were used for the detection of viruses in the sample concentrates. The suitability of these cells in the isolation of most viruses expected to be present in sewage has been demonstrated (24). Eagle minimal essential medium in Earle base (Autopow; Flow Laboratories) with glutamine and 10% fetal calf serum (Microbiological Associates) was routinely used for growing the cells. The serum concentration was reduced to 2% when minimal essential medium was used for the maintenance of cultures. The serum was screened for viruses and mycoplasma by the supplier. Gentamicin (Schering Corp.) was regularly added to growth and maintenance media at a final concentration of 50 μg/ml.

As stock cultures, the cells were cultivated in 75-cm^2 plastic tissue culture flasks (Falcon Plastics), and for sample inoculation they were grown in 25-cm^2 flasks.

Sample collection. The following three types of field samples were collected on a weekly basis from the Green Creek and Bilberry Creek plants in Ottawa: (i) raw sewage (RS), (ii) primary effluent before chlorination (EBC), and (iii) primary effluent after chlorination (EAC). Relevant information about the two plants is summarized in Table 1.

Because of the relatively small amounts (3.0×10^6 liters per day) of sewage being treated at the Bilberry Creek Plant, it was possible for us to collect from it temporally coordinated samples. About 200 ml of a

2.5% aqueous solution of uranine (Fisher Scientific) was added to sewage at the main inlet. This gave the sewage a bright, apple green color. A 5-liter sample of dye-containing RS was immediately withdrawn and collected. Following the dye tracer the sample of EBC was collected at the end of the sedimentation tank and that of EAC was collected at the end of the chlorine retention tank. Chlorine in the EAC was neutralized with a 1% aqueous solution of sodium thiosulfate.

The samples were collected between 9 and 11 a.m. and transported to the laboratory immediately for processing the same afternoon.

Sample processing. The talc-Celite technique, which has been described in detail elsewhere (23), was used for sample concentration throughout this study. In brief, the pH of a 5-liter volume of the sample to be processed was brought to 6.0; it was then passed through a talc-Celite layer, and elution from the layer was carried out with 50 ml of 10% fetal calf serum in saline (pH 7.2). The eluate was membrane (0.2 μm) filtered before inoculation into cell cultures.

The growth medium from BS-C-1 cultures (25 cm^2) was first poured off, and the monolayers were washed once with 2.0 ml of physiological saline. Five to 10 flasks were inoculated (0.5 ml per culture) with each sample concentrate to be tested, and they were incubated a 37°C for 1 h to allow for virus adsorption. At the end of this period, maintenance medium was added, and the cultures were placed back at 37°C.

The inoculated cultures were observed periodically over a 3-week period for virus cytopathology (CPE). Material from cultures showing CPE during this period was passed once in a fresh lot of cultures to confirm the presence of a viral agent. Those cultures that did not show any CPE at the end of the initial 3-week period were subjected to a blind passage. Absence of CPE in the second set of cultures was taken to indicate the absence in the sample concentrate of viruses detectable by the host system used.

Material from cultures showing CPE was examined under an electron microscope after negative staining with phosphotungstic acid. Only those virus isolates which appeared to be enteroviruses under an electron microscope were subsequently identified serologically (14). All poliovirus isolates were subjected to serodifferentiation (17) and temperature marker (16) tests.

The infectious units in virus-positive samples were estimated by the most probable number method (26).

RESULTS

A total of 282 field samples of RS, EBC, and EAC were examined for enteric viruses in this study. Sample collection from the Bilberry Creek Plant was commenced in May, 1974, and was continued up to April, 1976. All the samples from this plant, which represented 69% (195/282) of the total, were temporally coordinated. As can be seen from the results summarized in Table 2, viruses were detected in 80% (52/65) of RS, 71% (46/65) of EBC, and 58% (38/65) of EAC samples from this plant.

Sample collection from the Green Creek Plant

TABLE 1. *Relevant information on the Bilberry Creek and Green Creek sewage treatment plants in the Ottawa, Ontario, area*[a]

Treatment plant	Sewage flow (liters/day)	RS pH	RS 5-day BOD[b] (ppm)	Effluent 5-day BOD (ppm)	RS suspended solids (ppm)	Effluent suspended solids (ppm)	Suspended solids efficiency (%)	Chlorine residual (ppm)	Population served
Green Creek	3.67×10^8	7.5	79	48	114	52	55	0.5	4.0×10^5
Bilberry[c] Creek	3.0×10^6	7.7	98	44	146	41	72	0.5	3.0×10^3

[a] Data supplied by the Regional Municipality of Ottawa-Carleton.
[b] BOD, Biological oxygen demand.
[c] This plant has recently been phased out, and the sewage from this area is now being treated by the Green Creek Plant.

TABLE 2. *Detection of viruses in field samples of RS and effluents from two treatment plants in the Ottawa, Ontario, area*[a]

Plant	RS			EBC			EAC		
	No. positive/no. tested	% Positive	VIU/100 ml	No. positive/no. tested	% Positive	VIU/100 ml	No. positive/no. tested	% Positive	VIU/100 ml
Bilberry Creek[b]	52/65	80	100	46/65	71	100	38/65	58	2.7
Green Creek	23/29	79	ND[c]	22/29	76	ND	15/29	51	ND
Total	75/94	79.8		68/94	72.3		53/94	56.3	

[a] Samples (5 liters each) were concentrated by the talc-Celite technique (23), and concentrates were inoculated into BS-C-1 cells for virus isolation.
[b] All samples from this plant were temporally coordinated.
[c] ND, Not done.

was carried out from May, 1974, to April, 1975. Thirty-one percent of the samples tested in this study came from this plant. Because of the relatively large volumes of sewage being treated there, collection of temporally coordinated samples from this plant was found to be difficult. The results of virus isolations from these samples are also presented in Table 2. Seventy-nine percent (23/29) of RS, 76% (22/29) of EBC and 51% (15/29) of EAC samples from this plant were found to be virus positive.

Thirty-six percent (70/196) of the virus isolates obtained in this study were identified by electron microscopy and serology. These were found to represent the reo- (51.5%) and enterovirus (48.5%) groups. Polio-, echo-, and coxsackievirus B were among the identified enteroviruses. All poliovirus isolates were found to be vaccine strains when subjected to serodifferentiation (17) and temperature marker (16) tests. The identified isolates and field samples yielding them are presented in Table 3.

Estimations (26) of the amounts of infectious virus in representative temporally coordinated samples from the Bilberry Creek Plant showed that little or no virus removal was being achieved during primary treatment of sewage. On an average, the RS and EBC samples contained 100 viral infective units (VIU) per 100 ml. Reduction in the amount of virus on chlorination of the effluent varied between 10- and 50-fold. On the basis of these estimations and with a combined daily EAC output of 3.7×10^8 liters per day from these two plants, about 1.0×10^{10} VIU was being discharged into the river every day. With a mean annual river flow of 8.0×10^{10} liters per day (7), the discharged effluent was being diluted 216 times, resulting in a virus loading of approximately 1.0 VIU/8 liters of the river water.

TABLE 3. *Virus isolates identified by electron microscopy and serology and the field samples yielding them*

Virus type	No. of isolates in RS	No. of isolates in EBC	No. of isolates in EAC
Reoviruses	13	13	10
Enteroviruses			
Poliovirus type 1[a]	3	2	1
Poliovirus type 2[a]	3	4	1
Poliovirus type 3[a]	1	2	
Echovirus type 1	2	3	3
Coxsackievirus B2		1	
Coxsackievirus B3	1	2	1
Coxsackievirus B4		3	1

[a] All poliovirus isolates were found to be vaccine strains. All virus isolations were made by inoculation of sample concentrates into monolayer cultures of BS-C-1 cells.

DISCUSSION

Growth in the size of our urban centers is accompanied by an increase in the volume of liquid wastes being generated. Because in most places such wastes are discharged into the water environment after no or inadequate treatment, there has been a gradual deterioration in the quality of receiving waters (20). Ever greater reliance is being placed on these polluted sources to meet the increasing demands for potable and recreational waters. In Canada, for example, 90% of the urban population has sewage collection facilities, but liquid wastes generated by nearly half of the urban population are disposed of either raw or as chlorinated primary effluents (6). The inefficient nature of such treament at least in the removal and inactivation of enteric viruses is well documented (1, 8). This is further reinforced by the results of the present investigation. Viral pollution of surface waters due to chlorinated secondary effluent discharges has also been reported (9, 21).

Because of the interplay of a number of factors, the virus content of sewage not only varies considerably among various communities (8), but is also different at different times of the day within the same community (18). This, and the inherent differences in the sample concentration and virus detection systems, makes it very difficult to carry out meaningful comparisons between the results of similar investigations. Nevertheless, the virus concentration of 100 VIU/100 ml of raw sewage is lower than the estimates of 700 VIU/100 ml put forward earlier (13). Based on the latter estimation, the expected density of virus in sewage-polluted waters was calculated to be 0.15 to 1.5 VIU/100 ml (13). It must be noted here that the calculated virus loading of 1.0 VIU/8 liters of the Ottawa River water does not take into consideration viral pollution of the river through sources other than the chlorinated primary effluents. Other communities on the river discharge into it at least 9.0×10^7 liters of RS per day. This, together with undetermined but substantial amounts of storm waters and agricultural wastes, further adds to its virus load. At least 6.0×10^5 people depend on this river for their potable water. Beaches on it have remained closed for the past

few summers because of high levels of fecal pollution.

Primary treatment of sewage is also inefficient in the removal of organic impurities. Chlorination of effluents containing high levels of organic matter leads to the formation of products toxic to fish life (29). There is fear that these chemicals may be harmful to humans as well (15, 27). If chlorine is to be used for the terminal disinfection of sewage effluents without the generation of potentially harmful chemicals, it will be necessary to first remove most of the organic impurities from effluents. Additional treatment needed to accomplish this will most likely result in better virus elimination as well (2).

The talc-Celite technique was found highly satisfactory in the processing of field samples in this study. It must, however, be noted that from experimentally contaminated samples of sewage this technique was able to recover approximately 50% of the added virus (23). Sample concentrates obtained through this procedure were always free from cytotoxicity. Because a large proportion of viruses in sewage are particle associated (28), sample prefiltration was avoided here. Retention of the particulate matter on the talc-Celite layers made it possible to elute viruses associated with the solids as well as those adsorbed to the layers. However, keeping in view the limitations of the sample concentration and virus isolation systems, the amounts and types of viruses recovered here should be regarded as representing only a fraction of what might have been present in the samples tested.

It has been shown that enteric viruses discharged into the aquatic environment can be transported in an infectious state for several miles (21). Because water temperature appears to be one of the key factors in determining virus survival (22), climatic conditions in temperate areas would be highly conductive, for a good part of the year, to the retentions of viral infectivity in polluted surface waters.

Viruses have been isolated from treated surface waters (5, 11, 25), and outbreaks of waterborne diseases appear to be on the increase (4). Recreational waters have also been implicated in outbreaks of diseases due to hepatitis A (3) and enteroviruses (10). The continued disposal of ever increasing amounts of potentially dangerous wastes into the water environment is likely to create more serious health hazards. Therefore, the most logical approach to better water conservation as well as minimizing the possible health hazards would be to subject wastes to better treatment before their disposal.

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