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Survey of Human Enterovirus Occurrence in Fresh and Marine Surface Waters on Long Island

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A variety of surface water systems, including a lake, a creek, and two marine embayments, were analyzed on a monthly basis for indigenous human enteroviruses and coliform bacteria. Findings are discussed in terms of the probable pollution sources to each system and their relationship to data from previous studies.

Concern over the possible transmission of diseases of viral etiology through surface waters has recently been amplified by the growing interest in conserving and reclaiming the natural aquatic environment for recreational and economic purposes. The presence of human viruses in various surface waters has been documented by a number of investigators. Simkova and Wallnerova (30) isolated coxsackievirus from waters of the Danube River. Nestor and Costin (26) reported similar findings in sewage-contaminated river waters in Roumania. Human enteric viruses have been isolated in estuaries (20, 34), as well as in seawater and coastal marine sediments (4, 11). In the latter study (11), the concentration of viruses isolated from marine waters ranged from 0.1 plaque-forming unit (PFU) per 100 ml in moderately polluted waters, to 40 PFU/100 ml in heavily polluted waters located near sewage outfalls. The authors reported that viruses readily adsorbed to marine sediments and could be released into the water column by single mechanical shaking (similar to agitation occurring in natural waters).

The survival capacity of viruses in surface waters is highly variable and unpredictable (2). Although seawater has been shown to contain antiviral properties (17, 23, 36), constituents such as organic matter and particulates have been shown to be antagonistic to the action of nonspecific antiviral components, resulting in the extension of virus survival (3, 9, 23, 34). In addition, salinity and, more importantly, temperature have been shown to affect the survival of viruses in marine waters (15, 18).

The survival of enteric virus in nonmarine aquatic environments has not been studied extensively. Rhodes et al. (29) reported the extended survival of poliovirus in river water. Simkova and Wallnerova (31) demonstrated the ability of coxsackievirus A4 to survive for 45 days at a temperature of 22°C and for up to 154 days at 4°C in Danube River water. Herrmann et al. (12) found river and lake waters to be devoid of any inactivating capacity for poliovirus 1, and coxsackievirus A9 could be inactivated by water from a Wisconsin lake. The latter study demonstrated that inactivation occurred more rapidly in natural lake water than in sterilized lake water.

The occurrence of human virus (i.e., enterovirus) in various shellfish species is well documented. Morris et al. (24), while studying the presence of virus in the California mussel, found that 18 of 30 samples tested contained virus. The mussels had been taken from beds located near sewage outfalls which were discharging primarily and secondarily treated wastewater. Fugate et al. (8) found virus in 2 of 17 oyster samples in Texas and in 1 of 24 samples taken from the Louisiana Gulf Coast. The oysters had been taken from areas which met the approved coliform standard. Among viruses isolated were echovirus 4 and polioviruses 1 and 3. In 1968, Metcalf and Stiles (21) reported the isolation of poliovirus, coxsackievirus B3, and reovirus from shellfish growing in a sewage-polluted estuary in New Hampshire. Coxsackievirus A was isolated from oyster and mussel samples taken from a French market (5). Neutralization tests in suckling mice identified the majority of the French isolates as being coxsackievirus A16.

The present report describes the results of a year-long survey of virus occurrence in a variety of aquatic systems located on Long Island (New York), including a freshwater lake, a marine embayment receiving no direct discharge of domestic sewage and one of its tributary creeks, and a marine embayment into which treated

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wastewater effluents were released. The study, which involved sampling at monthly intervals, was part of a large-scale aquatic resources monitoring project sponsored by the federally funded Nassau-Suffolk "208" Wastewater Management Program.

MATERIALS AND METHODS

Sample sites. The following sites were selected for routine sampling.

(i) Freshwater inland lake (Lake Ronkonkoma). This site represents the largest freshwater body on Long Island. Although the surrounding area has undergone extensive residential and commercial development, there is no direct discharge of sewage to the lake waters. Virus sampling was conducted at a public bathing beach located on the western shore of the lake (Town of Islip). Sample volumes of 100 gallons (378.5 liters) each were taken from a depth of 5 feet (ca. 1.5 m) (approximately 15 feet [ca. 4.6 m] offshore) on a monthly basis between the months of June and September and at bimonthly intervals from October to May.

(ii) Brackish creek (Penataquit Creek). The creek, located on Long Island's South Shore (Town of Islip), is a tributary to Great South Bay. The banks of the creek contain numerous single- and multiple-family dwellings with individual septic systems. The lower portion of the creek is under considerable tidal influence from the bay. It was in this area of salt- and freshwater interface that samples (100 gallons) were taken monthly.

(iii) South shore embayment (Great South Bay). Sites for the collection of water (100 gallons) and occasional clam (100 to 200 g) samples included areas which had been designated as being "open" and "closed" to shellfishing by the New York State Department of Environmental Conservation. The closed area station was located 200 to 300 yards (ca. 183 to 274 m) below (south of) the discharge point of Penataquit Creek. Approximately 1 mile (ca. 1.6 km) south of this area was the open sampling station. Bay samples were collected at monthly intervals from June to September and March to May and at bimonthly intervals between October and February.

(iv) North shore embayment (Oyster Bay). The primary sampling station at this site was located within a mile of the outfall pipe of a secondary sewage treatment plant. Samples taken from this site included a 100-gallon water sample and occasional oyster samples. Sampling at the site was carried out with the same frequency as the south shore embayment. Treated effluent samples (of 25 gallons each [ca. 94.6 liters]) were taken from the nearby sewage treatment plant at monthly intervals to determine the likely contribution of this point source to viral pollution of the receiving waters.

Sample collection. Samples were collected in plastic, 55-gallon (ca. 208-liter) tanks (Plast-i-cube, Greif Brothers Corp.). Between collections, tanks were thoroughly rinsed with tap water, sanitized with 0.12 N hydrochloric acid for 30 min, and rinsed once again with tap water. Immediately before collection at each site, tanks were rinsed with 10 to 20 gallons (ca. 37.9 to 75.7 liters) of sample water before being filled. Pumping equipment (i.e., impeller pumps, hosing) was also rinsed with 10 to 20 gallons of sample water before collection. These precautions were taken to eliminate the chance of cross-contamination between samples.

Virus concentration procedures. (i) Water samples. Viruses in large-volume water samples were initially concentrated by means of an Aqualla virus concentrator (Carborundum Corp.). Sample volumes were first pumped through a series of prefilters to remove debris. Sample pH was then adjusted to 3.5, and aluminum chloride was added to a final concentration of 0.0005 M. The water then flowed through virus concentrating filters, which consisted of a fiber glass depth cartridge filter and an epoxy-fiber glass-asbestos microfilter (Cox). Elution of adsorbed virus was carried out with two-liter volumes of 0.1 M glycine at pH 11.5. Eluates were then neutralized to pH 7.5 in an equal volume of pH 2.0 glycine. The concentration procedures routinely yielded a final volume of four liters, which was reconcentrated in the laboratory with an inorganic flocculation procedure (7). After the addition of 10% fetal calf serum, samples were stored at −72°C to await assay.

(ii) Shellfish samples. Viruses in shellfish samples were extracted using a technique described by Sobsey et al. (32). Shellfish (clams and oysters) were shucked and placed in 100-g samples. After homogenization, samples were acidified, causing formation of a virus-containing precipitate which could be centrifuged and collected. Viruses were eluted from the precipitate with a glycine-saline solution and then separated from the rest of the precipitate by centrifugation. Virus-containing supernatants were then filtered through a series of serum-treated 47-mm membrane filters (0.8-, 0.45-, and 0.22-μm porosity, respectively) and concentrated by ultrafiltration to a final volume of 5 ml. Processed samples were frozen at −72°C awaiting tissue culture assay.

Virus isolation and identification. Viral enumerations from field samples were carried out on monolayers of low-passage buffalo green monkey kidney cells (Microbiological Associates) which were propagated on Eagle minimum essential medium with Hanks balanced salt solution and supplemented with 10% fetal calf serum and antibiotics (penicillin, streptomycin, gentamicin). Sample volumes of 0.5 ml were placed on cell monolayers in 25-cm^2 flasks and incubated for 2 h to facilitate virus attachment. After being decanted of excess sample material, cells were overlaid with 4 ml of neutral red agar medium (13) and incubated at 36°C under 5% CO_2 for 10 days. Daily readings were taken to determine the presence of viruses which appeared as plaques. After the incubation period, plaques were picked and enriched on monolayers of buffalo green monkey cells propagated in 24-well Cluster dishes (Costar). Isolates were identified in microtiter plates by serum neutralization techniques (19), using enterovirus typing pools.

Poliovirus T-marker studies. Isolates, identified as being members of the poliovirus group, were subjected to T-marker analysis to differentiate between vaccine strain and wild-type virus (14).

Coliform studies. To correlate virus data with a recognizable biological pollution indicator, total and

fecal coliform numbers were determined for all samples collected. Coliform enumerations were carried out using standard most-probable-number methods (28).

RESULTS

Lake waters. Viruses were recovered from lake waters during the months of September (1976) and March (1977), when corresponding coliform numbers were at low levels (Table 1). Additional summer (July, August) isolations may have been prevented by the presence of algal blooms which caused considerable clogging of virus concentrating filters.

The sampling station was located in a bathing area (Lake Ronkonkoma) used primarily by families with younger children. Bathers were the likely source of the 7 September 1976 isolates, which could not be specifically identified. The source of the 9 March 1977 isolates (poliovirus 2 [vaccine strain] and one unknown), however, was not immediately apparent.

Creek waters. Viral isolations were made in the waters of Penataquit Creek during June and July 1976 (Table 2). Isolates included coxsackieviruses A9 and B3 and echoviruses 2, 6, and 15 plus three unknown viruses (29 June 1976). Isolates on 15 July 1976 included echoviruses 25 and 32. Virus occurrence did not correlate well with coliform counts (Table 2), a finding which was not unexpected due to the large number of waterfowl (ducks, gulls) residing in the sampling area. The high coliform densities encountered during the month of August may have arisen from these nonhuman sources. It was of interest to note that poliovirus was not among species identified from creek samples.

Marine embayments. (i) South shore. The area of Great South Bay under study receives no direct discharges of treated wastewater. There are, however, numerous boats which release raw sewage into these waters during summer months. In addition, several tributary creeks, including the one previously described, discharge into the bay and may contribute to virus occurrence in the bay.

In terms of viral quality, there was little difference between the waters of the open and closed areas. Viruses were recovered from open waters during summer and early spring months and from closed areas during summer and late winter months (Tables 3 and 4). Limited shellfish sampling yielded virus isolates from open water clams in April (30 PFU/100 g) and June (10 PFU/100 g) of 1977 and from closed water clams during July 1976 (16 PFU/100 g) and June 1977 (10 PFU/100 g).

Virus isolates from water and shellfish included a variety of echovirus types, two vaccine

TABLE 1. *Coliform and virus isolations from Lake Ronkonkoma*

Isolation period	Total coliforms/100 ml	Fecal coliforms/100 ml	Virus PFU/gallon
July 1976	230	230	NI[a]
August 1976	2,300	930	NI
September 1976	43	43	2.3
October 1976	NT[b]	NT	NT
November 1976	930	930	NI
December 1976	NT	NT	NT
January 1977	14	9	NI
February 1977	NT	NT	NT
March 1977	7	NT	6.5
April 1977	NT	NT	NT
May 1977	150	75	NI

[a] NI, No isolates.
[b] NT, Not tested.

TABLE 2. *Coliform and virus isolations from Penataquit Creek*

Isolation period	Total coliforms/100 ml	Fecal coliforms/100 ml	Virus PFU/gallon
June 1976	43,000	43,000	25.0
July 1976	1,100	460	8.0
August 1976	230,000	9,300	NI[a]
September 1976	NT[b]	NT	NT
October 1976	9,300	2,300	NI
November 1976	1,500	390	NI
December 1976	930	93	NI
January 1977	9,300	4,300	NI
February 1977	9,300	NT	NI
March 1977	15,000	NT	NI
April 1977	4,300	4,300	NI
May 1977	4,300	430	NI

[a] NI, No isolates.
[b] NT, Not tested.

TABLE 3. *Coliform and virus isolations from Great South Bay open shellfish waters*

Isolation period	Total coliforms/100 ml	Fecal coliforms/100 ml	Virus PFU/gallon
July 1976	4	4	8.0
August 1976	460	4	1.2
September 1976	93	<3	NI[a]
October 1976	NT[b]	NT	NT
November 1976	43	NT	NI
December 1976	NT	NT	NT
January 1977	NT	NT	NT
February 1977	93	NT	NI
March 1977	23	<3	NI
April 1977	150	15	2.9
May 1977	NT	NT	NT
June 1977	93	<3	NI

[a] NI, No isolates.
[b] NT, Not tested.

strains of poliovirus, and numerous viruses which could not be identified (Tables 5 and 6).

(ii) North shore. Viruses were frequently isolated from the treated wastewater effluents being discharged into Oyster Bay (Tables 7 and 8). Viral concentrations ranged from 67.2 to 2,636.4 PFU/gallon. The highest virus numbers were encountered during the month of March when enteroviral numbers would be expected to be at their lowest. This result could not be readily explained. Effluent samples from July (Table 7) were quite turbid, which may have accounted for the apparent inability to remove coliforms by chlorination.

TABLE 4. *Coliform and virus isolations from Great South Bay closed shellfish waters*

Isolation period	Total coliforms/100 ml	Fecal coliforms/100 ml	Virus PFU/gallon
July 1976	430	75	4.0
August 1976	110	23	NI[a]
September 1976	93	4	NI
October 1976	NT[b]	NT	NT
November 1976	2,300	43	NI
December 1976	NT	NT	NT
January 1977	NT	NT	NT
February 1977	150	NT	4.4
March 1977	45	15	NI
April 1977	2,400	460	NI
May 1977	NT	NT	NT
June 1977	23	4	1.1

[a] NI, No isolates.
[b] NT, Not tested.

TABLE 5. *Virus isolate identifications from Great South Bay open water and shellfish*

Date	Sample type	Identifications
7 July 1976	Water	U[a]
		U
		Poliovirus 2 (vaccine strain)
		Echovirus 22
		Echovirus 11
18 August 1976	Water	U
25 April 1977	Water	U
25 April 1977	Shellfish	U
2 June 1977	Shellfish	U

[a] U, Identity unknown.

TABLE 6. *Virus isolate identifications from Great South Bay closed waters and shellfish*

Date	Sample type	Identifications
7 July 1976	Water	U[a]
29 July 1976	Shellfish	Echovirus 20
		Echovirus 23
28 February 1977	Water	Poliovirus 2 (vaccine strain)
2 June 1977	Water	Poliovirus 1 (vaccine strain)
2 June 1977	Shellfish	Poliovirus 1 (vaccine strain)

[a] U, Identity unknown.

TABLE 7. *Coliform and virus isolations from Oyster Bay sewage treatment plant*

Isolation period	Total coliforms/100 ml	Fecal coliforms/100 ml	Virus PFU/gallon
June 1976[a]	4,300,000	390,000	NI[b]
July 1976	2,300,000	430,000	227.0
August 1976	23	<3	NT[c]
September 1976	430	43	67.2
October 1976	43	<3	NI
November 1976	9	<3	NI
December 1976	430	<3	NI
January 1977	39	<3	NI
February 1977	13	<2	NI
March 1977	150	NT	2,636.4
April 1977	2,300	NT	216.4
May 1977	23	<3	NI

[a] Unchlorinated.
[b] NI, No isolates.
[c] NT, Not tested.

TABLE 8. *Virus isolate identifications from Oyster Bay sewage treatment plant*

Date	Identifications
12 July 1976	Echovirus 25
	Echovirus 14
	Coxsackievirus A16
	Coxsackievirus B3
	Echovirus 17
	Echovirus 27
	Coxsackievirus B6
	Echovirus 11
	Echovirus 13
	Coxsackievirus A7
	Coxsackievirus B4
21 September 1976	Echovirus 5
	Echovirus 25
	Coxsackievirus B2
	Echovirus 17
	Echovirus 11
	Coxsackievirus B5
	Echovirus 6
	Poliovirus 3 (vaccine strain)
	Echovirus 12
8 March 1977	Coxsackievirus B3
	Echovirus 11
	Poliovirus 2 (vaccine strain)
5 April 1977	U[a]
	Echovirus 6
	Coxsackievirus B3
	Coxsackievirus A17

[a] U, Identity unknown.

In spite of the high frequency of the discharge of significant numbers of virus into the bay, positive isolations were made only from a single sample of the waters (Table 9). Isolates could not be identified using the National Institute of Allergy and Infectious Diseases serum pools. Coliform counts also tended to be low, but, once again, little correlation could be made with viral numbers. Viruses (48 PFU/100 g) were recovered from oyster samples during the month of March (1977).

The infrequency with which viruses were isolated from this area was likely related to the virucidal properties of the water, in conjunction with the distance between the sampling station and the sewage outfall.

DISCUSSION

Recent developments in virus concentrator technology have facilitated the isolation of human viruses from large volumes of surface water (7). However, the variable conditions presented during field sampling (e.g., salinity, turbidity, presence of planktonic blooms, etc.) tend to compromise the efficiency of the concentration process. Concentration and analytical methods used during this study were specifically designed to assess the occurrence of human enteroviruses. It was, therefore, not possible to determine the presence of other human virus groups (e.g., adenovirus, reovirus) which have also been isolated from surface waters (6, 21). As a result, data presented in this report likely represent the minimum number of human viruses actually present in each sample.

Little information is currently available regarding the isolation of human viruses in lakes. The present study describes the occurrence of human viruses in lake waters in two of the seven samples analyzed. Virus isolations noted in early September were attributed to the influence of bathers, and it is possible that viruses could have survived through the winter, resulting in the isolations recorded in March. This hypothesis is supported by the work of Simkova and Wallnerova (31), who reported that certain enteroviruses could survive in freshwater for periods of up to 5 months at 4 to 8°C. Gerba et al. (10) indicated the extended survival of viruses trapped within marine sediments. Although the possibility of long-term survival exists, it would be impractical to ignore the possible contributions of other potential sources to the lake such as seepage from nearby septic systems and storm water runoff.

TABLE 9. *Coliform and virus isolations from Oyster Bay open shellfish waters*

Isolation period	Total coliforms/100 ml	Fecal coliforms/100 ml	Virus PFU/gallon
July 1976	1,100	9	2.8
August 1976	230	93	NI[a]
September 1976	930	43	NI
October 1976	NT[b]	NT	NT
November 1976	23	23	NI
December 1976	NT	NT	NT
January 1977	NT	NT	NT
February 1977	23	NT	NI
March 1977	4	NT	NI
April 1977	<3	<3	NI
May 1977	NT	NT	NT
June 1977	15	<3	NI

[a] NI, No isolates.
[b] NT, Not tested.

Several authors have demonstrated the presence of human viruses in the tributary waters of marine embayments and coastal waters (11, 20, 33). In these studies, pollutants could be traced to the wastewater discharges of sewage treatment plants. In our study of Penataquit Creek, the virus isolations could not be traced to such discharges. The likely sources of viral pollution to the area were the seepage from septic systems located along the banks of the creek and the discharge of raw sewage from boats. The precise relationship of these potential sources to viral occurrence in similar aquatic systems has not been fully investigated.

Numerous investigators have described the isolation of human viruses from shellfish and shellfish-growing waters (4, 5, 8, 21, 33). The present Great South Bay survey indicated little virological difference between areas designated as open and closed to shellfishing on the basis of coliform counts. These findings, from sampling stations located approximately 1 mile apart, do not conflict with previous virus survival studies which have shown extended survival in marine estuarine waters, shellfish, and sediments (1, 2, 4, 22, 34). As effluents of sewage treatment plants were not released into this area, the likely contributors of viral contamination were the various tributaries (e.g., Penataquit Creek) in the area and, to a lesser extent, sewage discharges from boats.

Viral sources to the north shore embayments may have included septic tank leachate and storm water runoff, but these potential sources were overshadowed by the virus content of the wastewater effluents being discharged into the system. Previous embayment studies by Metcalf et al. (22) demonstrated the presence of viruses several miles from the nearest sewage outfall. In the present study, viruses were recovered from the waters and shellfish of a sampling station located within 1 mile of a known virus source on a single occasion. Determination of the mecha-

nisms influencing virus removal could not be carried out within the constraints of this survey, but processes similar to those reported by others were likely involved (16, 27, 36).

With the exception of hepatitis (25), there is little epidemiological information presently available concerning the waterborne disease potentials of human viruses. Should a relationship between viruses in water and disease outbreaks be unequivocally established by future study, water quality standards would require adjustment to include indices for virus presence. Currently practiced methods for the determination of microbial quality of water (i.e., total and fecal coliform counts) have been shown to be inapplicable to viruses by this and previous studies (8, 33, 35). Therefore, health officials contemplating the need for viral quality assessment in waters should consider the utilization of a human virus index, in lieu of a system based upon the enumeration of a surrogate bacterial organism.

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