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Survey of Human Virus Occurrence in Wastewater-Recharged Groundwater on Long Island†

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Treated wastewater effluents and groundwater observation wells from three sewage recharge installations located on Long Island were assayed on a monthly basis for indigenous human enteroviruses and coliform bacteria for a period of 1 year. Viruses were detected in groundwater at sites where recharge basins were located less than 35 feet (ca. 10.6 m) above the aquifer. Results from one of the sites indicated the horizontal transfer of viable viruses through the groundwater aquifer.

The increasing demand for potable water to supply domestic and commercial needs has prompted a search for a means of supplementing freshwater reserves. Among methods proposed are those dealing with the recharge of groundwater aquifers with renovated wastewater, including spray irrigation, land application, deep-well injection, and basin recharge. Inherent in any scheme of wastewater reuse is the potential hazard posed by the presence of human viruses commonly associated with sewage (1, 9).

Several studies have described the various factors affecting virus removal during wastewater recharge through soils. Drewry and Eliassen (6) indicated adsorption rather than filtration to be the probable mechanism of virus removal during sand or soil percolation. Gerba et al. (9) reported that the adsorption process was strongly influenced by a number of factors, including the pH of recharged water, chemical composition and moisture content of the soil, and the rate of recharge. The ionic strength of the adsorbing environment was also shown to be an important factor in the attachment of virus to soil particles (7, 14, 22). Clean, dry sand was shown to have little removal capabilities (2), whereas moistened sand demonstrated an improved removal efficiency (18). Drewry and Eliassen (6) reported that soils having a high clay and silt content were the most effective virus adsorbents. Clay particles were shown to be excellent adsorbents due to their large surface area (3).

Although soil adsorption mechanisms have been the subject of a number of reports, comparatively few have described studies of naturally occurring viruses at operational recharge installations. Wellings et al. (22) reported three separate isolations of poliovirus and coxsackievirus from groundwater beneath a cypress dome being used for the recharge of secondarily treated effluent. Schaub and Sorber (20) also demonstrated the sporadic occurrence of enterovirus in recharged groundwater. Gilbert et al. (10), however, were unable to detect viruses in six groundwater samples taken from the Flushing Meadows recharge project.

The present report describes the results of a year-long virus survey conducted at three wastewater recharge facilities located on Long Island. The study, which involved sampling at monthly intervals, was part of a large-scale, aquatic resource-virus-monitoring project sponsored by the federally funded Nassau-Suffolk "208" program.

MATERIALS AND METHODS

Sampling sites. A total of three sewage treatment plants (STP) were selected for this study. Recharge operations at each had been in progress for several years. The following sites were included.

(i) Meadowbrook STP, located in East Meadow, N.Y. (Nassau County), included a 1,000,000-gallon-per-day-capacity (3,785,000 liters) secondary sewage treatment plant (trickling filter) which served the hospital complex of the Nassau County Medical Center and the Nassau County Jail. Adjacent to the treatment facility was a series of recharge basins into which chlorinated effluents were discharged. Each basin was located approximately 30 feet (ca. 9 m) above the groundwater aquifer, and was primarily composed of coarse sand and fine gravel, with a 1 to 2% silt content. An observation well was located 10 feet (3 m) down gradient from the basins. The well was driven into the upper surface of the aquifer (approximately 37 feet [ca. 11.3 m]). Samples collected at monthly intervals included a 25-gallon (ca. 94.6-liter) volume of chlorinated STP effluent and 100 gallons (378.5 liters) of observation well water.

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(ii) Stony Brook STP, located in the village of Stony Brook (Suffolk County), served a housing development. The site contained a 300,000-gallon-per-day-capacity (1,135,000 liters) secondary treatment plant (contact stabilization), with disinfection by chlorination, and a series of recharge basins into which effluents were discharged. The basins, located 80 feet (ca. 24.4 m) above the water table, consisted of coarse sand and fine gravel with approximately 2% silt. Observation wells, drilled to depths of 84 feet (25.6 m), were located 8 feet (ca. 2.4 m) down gradient from the basins. Samples were collected from the treatment plant (25 gallons) and observation well (100 gallons) at monthly intervals.

(iii) Parkland III STP, located at Holbrook, N.Y. (Suffolk County), served a moderate-sized housing development. The facility included a 260,000-gallon-per-day-capacity (984,100 liters) tertiary treatment plant (extended aeration, denitrification, gravity sand filtration) with recharge basins constructed 18 feet (ca. 5.5 m) above groundwater. Soils in the basins were similar to those previously described. An observation well driven to a depth of 20 feet (ca. 6.1 m) was located 50 yards (45.7 m) down gradient from the basins. Sample volumes were the same as previously mentioned.

Sample collection. Samples were collected in plastic 55-gallon (ca. 208.2-liter) tanks (Plast-i-cube, Greif Brothers Corp.). Between collections, tanks were thoroughly rinsed with tap water, sanitized with 0.12 N hydrochloric acid (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 65.7 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 obviate the chance of cross-contamination between samples.

Virus concentration procedures: water samples. Viruses in large-volume water samples were initially concentrated by means of an Aquella virus concentrator (Carborundum Corp.). Appropriate sample volumes were 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 sandwich (0.45 and 1 μm, Cox AA45 and AA100). Elution of adsorbed virus was carried out with 2-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 4 liters, which was reconcentrated in the laboratory by means of an inorganic flocculation procedure (8) to a final volume of 25 to 100 ml. After the addition of 10% fetal calf serum, samples were stored at −72°C to await assay.

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 grown on minimum essential medium with Hanks balanced salt solution and 10% fetal calf serum. Sample volumes (0.5 ml) were placed on cell monolayers in 25-cm^2 flasks (5 to 10 flasks per sample) and incubated for 2 h to facilitate virus attachment. After excess sample material was decanted, cells were overlaid with 4 ml of neutral red agar medium (17) and incubated at 36°C under 5% CO_2 for a period of 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 kidney cells propagated in 24-well cluster dishes (Costar). Isolates were identified in microtiter plates by serum neutralization techniques (16), 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 (13).

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 (11).

RESULTS

Viruses were isolated from the chlorinated effluents of the Meadowbrook STP on three occasions (Table 1). Little correlation was noted between virus and coliform occurrences in these samples. It is possible that viruses were present in those samples with very high coliform densities (August and January), but their adsorption and/or elution from virus-concentrating filters may have been compromised by the high turbidity of the effluents which occurred on those particular days. Virus types isolated from effluent samples included a number of echo-, coxsackie-, and polioviruses and several isolates which could not be identified (Table 2). Low numbers of virus were isolated on three occa-

TABLE 1. *Coliform and virus isolations: Meadowbrook STP*

mo and yr	Total coliforms/100 ml	Fecal coliforms/100 ml	Virus PFU[a]/gallon
June 1976	430,000	23,000	80.0
July 1976	23,000	9,300	NI[b]
August 1976	750,000	43,000	NI
September 1976	<3	<3	6.4
October 1976	230	<3	NI
November 1976	230	<3	NI
December 1976	2,300	43	NI
January 1977	11,000,000	2,400,000	NI
February 1977	49	11	100.0
March 1977	9,300	NT[c]	NI
April 1977	9,300	NT	NI
May 1977	2,300	4	NI

[a] PFU, Plaque-forming units.
[b] NI, No isolates.
[c] NT, Not tested.

sions from the observation well (Table 3), indicating the ability of the organisms to move vertically through the basin. The likelihood of horizontal movement of viruses through the aquifer could not be ascertained due to the close proximity of the observation well to the recharge basin (8 feet). At this distance the well likely drew from the dome of recharged water extending outward from beneath the basin.

Enteroviruses were isolated from the Stony Brook treatment facility during the winter and early spring months (Tables 4 and 5). Observation well samples yielded no virus isolations (Table 6), indicating the apparent inability of viruses to penetrate the 80-foot depth from basin bottom to aquifer. In general, coliform numbers from basin samples were quite low. The sharp increases noted in the December samples could not be readily explained.

The tertiary treatment plant under study (Parkland III) experienced a number of operating difficulties during the study period. The most notable result of these difficulties was the appearance of a significant number of viral isolates and high colifom counts from treated ef-

TABLE 2. *Virus isolate identifications: Meadowbrook STP and observation well*

Date	Sample type	Identifications included:
22 June 1976	Effluent	Echovirus type 13 Echovirus type 21 Coxsackievirus type B-3
17 August 1976	Observation well	Echovirus type 12 U[a]
13 September 1976	Effluent	Coxsackievirus type B-4 Coxsackievirus type B-3 Echovirus type 6 Poliovirus type 1 (vaccine strain)
13 September 1976	Observation well	U
2 February 1977	Effluent	Coxsackievirus type B-4 Echovirus type 30 U
5 April 1977	Observation well	U

[a] U, Identity unknown.

TABLE 3. *Coliform and virus isolations: Meadowbrook observation well*

mo and yr	Total coliforms/100 ml	Fecal coliforms/100 ml	Virus PFU[a]/gallon
August 1976	23,000	4	1.3
September 1976	23	15	3.6
October 1976	23	9	NI[b]
November 1976	430	23	NI
December 1976	23	<3	NI
January 1977	4,300	150	NI
February 1977	27	<2	NI
March 1977	39	NT[c]	NI
April 1977	15	NT	2.4
May 1977	NT	NT	NI

[a] PFU, Plaque-forming units.
[b] NI, No isolates.
[c] NT, Not tested.

TABLE 4. *Coliform and virus isolations: Stony Brook STP*

mo and yr	Total coliforms/100 ml	Fecal coliforms/100 ml	Virus PFU[a]/gallon
June 1976	7,500	3,900	NI[b]
July 1976	2,300	150	NI
August 1976	9	<3	NI
September 1976	9,300	<30	NI
October 1976	4	<3	NI
November 1976	11,000,000	NT[c]	84.4
December 1976	2,400,000	430,000	369.6
January 1977	2,000	23	NI
February 1977	9,300	430	NI
March 1977	4,300	NT	32.4
April 1977	930,000	240,000	23.2
May 1977	240,000	240,000	NI

[a] PFU, Plaque-forming units.
[b] NI, No isolates.
[c] NT, Not tested.

TABLE 5. *Virus isolate identifications: Stony Brook STP*

Date	Identifications included:
9 November 1976	Echovirus type 2 Echovirus type 21 U[a] Coxsackievirus type A-16 Coxsackievirus type B-3
13 December 1976	Coxsackievirus type B-3 Poliovirus type 1 (vaccine strain)
16 March 1977	Echovirus type 6
12 April 1977	U

[a] U, Identity unknown.

TABLE 6. *Coliform and virus isolation: Stony Brook well*

mo and yr	Total coliforms/100 ml	Fecal coliforms/100 ml	Virus PFU[a]/gallon
September 1976	4	<3	NI[b]
October 1976	4,300	7	NI
November 1976	430	NT[c]	NI
December 1976	23,000	930	NI
January 1977	43	4	NI
February 1977	390	<3	NI
March 1977	93	NT	NI
April 1977	NT	NT	NI
May 1977	150	<3	NI

[a] PFU, Plaque-forming units.
[b] NI, No isolates.
[c] NT, Not tested.

fluents (Table 7). Among the viruses identified from these samples were strains of poliovirus types 2 and 3 that were capable of undergoing replication at 40°C; these were tentatively identified as being nonvaccine strains (Table 8). Despite the high influx of viruses and bacteria to the recharge basins, comparatively low numbers were isolated at lower frequency from observation well samples (Table 9). Since this sampling well was located some 50 yards down gradient from the basins, the data provided evidence of both vertical penetration of viruses through the basin and their horizontal entrainment through the aquifer for an appreciable distance.

TABLE 7. *Coliform and virus isolations: Parkland III STP*

mo and yr	Total coliforms/100 ml	Fecal coliforms/100 ml	Virus PFU[a]/gallon
July 1976	430	3	NI[b]
August 1976	4	<3	NI
September 1976	75,000	430	6.8
October 1976	930	15	NI
November 1976	430,000	430	NI
December 1976	930,000	4,300	22.0
January 1977	11,000,000	23,000	94.7
February 1977	23,000	230	315.5
March 1977	230,000	NT[c]	1,070.7
April 1977	2,400,000	93,000	94.0
May 1977	NT	NT	NT
June 1977	2,400	2,400	NI

[a] PFU, Plaque-forming units.
[b] NI, No isolates.
[c] NT, Not tested.

TABLE 8. *Virus isolate identifications: Parkland III STP and observation well*

Date	Sample type	Identifications included:
17 August 1976	Observation well	Echovirus type 6
6 September 1976	Effluent	Echovirus type 9
14 December 1976	Effluent	U[a]
14 December 1976	Observation well	Echovirus type 21
		Echovirus type 24
18 January 1977	Effluent	Poliovirus type 3 (vaccine strain)
		U
8 February 1977	Effluent	Coxsackievirus type B-3
		Poliovirus type 3 (nonvaccine strain)
		Poliovirus type 2 (vaccine strain)
8 February 1977	Observation well	U
		Echovirus type 25
		U
15 March 1977	Effluent	U
		Poliovirus type 2 (nonvaccine strain)
		Echovirus type 13
		Echovirus type 25
		Poliovirus type 3 (vaccine strain)
		Coxsackievirus type A-16
13 April 1977	Effluent	U
		Poliovirus type 3 (nonvaccine strain)
		Echovirus type 32

[a] U, Identity unknown.

DISCUSSION

The use of recently developed virus-concentrating units has greatly facilitated the isolation of human viruses from large volumes of water. However, the variability of conditions encountered during field sampling (e.g., turbidity, presence of organics, variations in ion content, etc.) tend to obviate a 100% efficiency of virus concentration. As a result, the data presented above likely represent the minimum numbers of virus in each sample. The inability to detect viruses within the constraints of our testing systems could not preclude the possibility of viral presence in low concentrations.

Currently practiced sewage treatment methods cannot guarantee the removal of all human viruses, and their isolation from treated wastewater effluents has been the subject of numerous reports (4, 5, 15, 19, 21). The presence of these organisms has been viewed as a potential health hazard to wastewater reuse operations, especially those involving groundwater recharge (1, 2, 12). The practical application of many such schemes, therefore, could depend largely upon their ability to return relatively virus-free waters to the aquifer. To date, relatively few field studies have been carried out which addressed the question of naturally occurring viruses in wastewater recharge systems (10, 20, 22).

The present study, though survey in nature, provides some interesting information concern-

TABLE 9. *Coliform and virus isolations: Parkland III well*

mo and yr	Total coliforms/100 ml	Fecal coliforms/100 ml	Virus PFU[a]/gallon
August 1976	430	43	3.7
September 1976	930	43	NI[b]
October 1976	750	23	NI
November 1976	93	<3	NI
December 1976	430	9	1.6
January 1977	43	<3	NI
February 1977	15	<3	10.6
March 1977	4	NT	NI
April 1977	75	<3	NI
May 1977	NT	NT	NT
June 1977	460	150	NI

[a] PFU, plaque-forming units.
[b] NI, No isolates.
[c] NT, Not tested.

ing the virus removal capabilities of several ongoing recharge operations. The most promising results were obtained from the Stony Brook installation, where we were unable to isolate viruses from any of the eight samples tested. The recovery of viruses from groundwater at the Parkland III and Meadowbrook sites established the ability of virus particles to penetrate these shallower basins (18 and 34 feet, respectively). Gilbert et al. (10) were unable to detect viruses in the aquifer at the Flushing Meadows project. The apparent discrepancy between their findings and those resulting from our study might be explained by differences in the soil characteristics occurring in the areas studied.

Although effluent quality and soil conditions at each site were not identical, the data would seem to support the use of basins having appreciable depths to groundwater. The need for preventing the introduction of virus particles into groundwater was underscored by the Parkland III data, which indicated the apparent transport of viable viruses from treated wastewater through groundwater aquifers. Since little information is presently available on the survival of human viruses which have become entrained in aquifers, the implications of this finding cannot be determined as yet.

Though poliovirus occurred in the STP effluents, we were unable to detect it in any of the observation well samples. This finding raises some interesting questions concerning the comparative adsorptive properties among related viruses in a variety of soil-oriented processes, specifically, the use of seeded poliovirus experiments to predict the fate of other members of the enterovirus group under laboratory-manipulated conditions.

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