APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1983, p. 1474–1480
0099-2240/83/051474-07$02.00/0
Vol. 45, No. 5

Entrainment of Viruses from Septic Tank Leach Fields Through a Shallow, Sandy Soil Aquifer

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Received 17 November 1982/Accepted 8 March 1983

A study was conducted which focused on movement of naturally occurring human enteroviruses from a subsurface wastewater disposal system through a shallow aquifer. The potential for significant entrainment of virus particles was evidenced by their recovery at down-gradient distances of 67.05 m and from aquifer depths of 18 m. A significant negative correlation was observed between virus occurrence and the distance from the "septage" (leaching pool) source. Virus occurrence could not be statistically correlated with either total or fecal coliforms, indicating the limitations of current microbial water quality indicators for predicting the virological quality of groundwater.

The past several years have witnessed the development of considerable information concerning the ability of human viruses to penetrate soil and contaminate underlying shallow groundwater systems (7, 16, 21–24). Given the potential for virus contamination of an aquifer by sewage disposal (e.g., subsurface disposal systems, wastewater recharge basins), it is vital that information concerning the extent of viral entrainment in groundwater be developed. The need for such information is particularly acute in areas such as Long Island where sewage-contaminated aquifers may serve as the sole source of potable water for local residents.

The earliest evidence for the presence of viruses in groundwater, based upon virus recovery rather than epidemiological evidence, was provided in 1972 by Mack et al. (10). They isolated poliovirus type 2 from a 30.5-m-deep drinking-water well located 91.5 m from the edge of a wastewater drain field. The data suggested both vertical penetration of soil and significant lateral virus movement through the aquifer. Similar findings were later reported by Wellings (22), who described soil penetration and aquifer entrainment of viruses resulting from the discharge of secondarily treated wastewater through a cypress dome. In a later study, Wellings et al. (25) recovered echovirus 22/23 from a well located 30 m from a wastewater disposal site. Vaughn et al. (21) noted sporadic isolations of enteroviruses from two rapid wastewater infiltration sites located on Long Island. At the first site, echovirus type 12 as well as several unidentifiable isolates were recovered from a groundwater observation well adjacent (3.3 m) to a sand recharge basin receiving daily applications of secondarily treated wastewater. Virus recoveries at the second site provided additional evidence for lateral movement in the aquifer. Here, viruses were recovered from an observation well located 45.7 m down-gradient from recharge basins which received tertiarily treated effluents (20). Schaub and Sorber (15), studying groundwater beneath rapid wastewater infiltration basins, recovered viruses at soil depths of 30 m and at lateral distances of 182.8 m. Koerner and Haws (8), observing the results of rapid infiltration of primary effluent through coarse gravel and sand, recovered virus from groundwater at soil depths of 16.8 m and down-gradient distances of 250 m.

The above data clearly demonstrate virus entrainment as a result of surface disposal. However, few studies have specifically focused on vertical and lateral movement of viruses emanating from subsurface disposal systems. Vaughn and Landry (19) isolated unidentifiable virus types from a subsurface leaching pool which received secondarily treated effluents. In the same account, they reported a single isolation of coxsackievirus B3 from an observation well located some 402-m down-gradient from a sanitary landfill. Marzouk et al. (11, 12) isolated enteroviruses in 20.2% of groundwater samples tested in Israel. Although the precise source of sewage contamination was not identified, the authors suggested the likely sources as being subsurface seepage from septic tanks and underground sewer lines. Wellings et al. (25) demonstrated the presence of echovirus in a series of 10.6- to 12.1-m-deep groundwater wells which were clustered in an area surrounded by septic tanks. A similar virus source was proposed by Vaughn and Landry (19) after the isolation of echovirus types 11 and 23, and coxsackievirus

A-16 from a groundwater observation well located in the center of a cluster of single family dwellings which discharged wastes to individual septic systems.

The present study was designed to assess the lateral transport of indigenous human viruses through a shallow sole-source aquifer which received discharges from a subsurface disposal system. The study focused on the likely contribution of such systems to shallow aquifer contamination, and their potential impact on nearby drinking-water supplies.

MATERIALS AND METHODS

Site selection and well installation. The site chosen for the study was the Hampton Gateway apartment complex, Speonk, N.Y., located on the south shore of eastern Long Island. The complex consisted of three buildings, each containing 40 apartment units. Since the original plans had called for the eventual construction of three additional buildings (80 units), the disposal system (Fig. 1) had been designed to accommodate six apartment buildings, serving approximately 300 people. The system consisted of a 5.7×10^4-liter septic tank, three distribution pools (4.25 m^3), and 40 leaching pools (4.95 m^3). Each pool was installed approximately 3 m below grade, which resulted in a 3.6-m unsaturated zone above the static groundwater level. As the system operated at approximately one-half capacity, peripheral pools received little flow from the central septic tank. The major leachate plume was found to be concentrated around the central pools. Plume and groundwater-gradient measurements were conducted by the Groundwater Resources Section of the Suffolk County Department of Health Services. Their investigations showed that the groundwater flow was south 40° west, with a hydraulic gradient of 2.22 m/1.61 km. The configuration of the leachate plume was determined on the basis of physical and chemical measurements made on groundwater samples taken from eight test wells driven down-gradient from the leaching pools. Measurements included conductivity, ammonia, nitrate, nitrite, and chloride determinations. Subsequent data indicated that the septage percolate was concentrated down-gradient from the center of the sewage disposal system, moving slightly away (south) from the hydraulic gradient. On the basis of these examinations, a series of monitoring wells was driven along the line of predicted plume movement. Using the center of the system as the point of origin, wells (5.08-cm diameter) were driven at distances of 1.52, 3.05, 4.57, 7.62, 10.67, 15.24, 22.86, 30.48, 45.72, and 60.35 m. An additional well was driven 5 m north of the central pool array to serve as an up-gradient control. Each well was fitted with steel piping, having a 0.91-m stainless steel screen section set 1.52 to 1.83 m below the top of the water table. Also tested during the study was an existing drinking-water well located 67.05 m (18.29-m depth) down-gradient from the septage source. Sample water from this well was collected from a tap located on the side of one of the apartment buildings.

As seen in Fig. 1, a sewer line serving building number 3 crossed the study area between the 15.24- and 22.86-m monitoring wells. Since any virus isolations from these wells could be questioned on the basis of possible leaking from cracks or defective joints, the Health Department provided a detailed television inspection of the sewer line to determine its status. In addition to optical inspection, each joint was individually pressure tested (testing conducted by PENGAT Contracting Corporation, Setauket, N.Y.). All joints in the vicinity of the testing wells were found to be intact. Inspection of the entire pipe indicated no other defects at any point.

Field sampling. Sample collection. Groundwater samples (378 liters) were collected from each sample well at biweekly intervals during the initial phase of the study. Samples were collected in 209-liter acid-sterilized tanks (Plasti-Cube; Grief Brothers Corp., Staten Island, N.Y.) which had been arranged in permanent sets for exclusive use at each sample well. Pumping equipment and hosing (also arranged in sets for certain well groupings) were acid sterilized before each collection and thoroughly rinsed with 50 to 100 liters of sample water before the filling of the tanks. Bi weekly, 38-liter septage samples were also collected in containers used exclusively for this sample type. The above measures (plus others described below) were undertaken to avoid the likelihood of cross-contamination between different sample types.

Virus concentration and enumeration. Concentration of viruses from large-volume samples involved a filter adsorption-beef extract elution-organic flocculation method previously described by Landry et al. (9). Briefly, samples were acidified to pH 3.5, supplemented with 0.5 mM $AlCl_3$, and passed through a virus-concentrating filter series consisting of a fiberglass depth cartridge filter (K-27) and a pleated cartridge filter (Duo Fine, Timonium, Md.). Viruses were eluted from concentrating filters with 1-liter volumes of 3% beef extract–0.5 mM Tris (pH 9.5). Virus reconcentrations from 1-liter eluates followed the technique of Katzenelson et al. (6). Eluate pH was adjusted to 3.5, causing the formation of a virus-adsorbing protein precipitate. After 30 min, the percipitate was collected by centrifugation (5,000 × g for 10 min), and the resulting pellet was dissolved in 10- to 25-ml volumes of 0.15 M dibasic sodium phosphate (pH 9.0). Concentrates were then neutralized to pH 7.2 and stored at −60°C to await assay.

Virus concentration units were acid sterilized between each sample processing. Sterilization was accomplished by filling all portions of each system (hoses, filter holders with appropriate filters, pumps) with 1.2 N HCl and allowing a 30-min contact period. Systems were then thoroughly rinsed with tap water and appropriate sample water. Two separate units were constructed for use in the study, one for the near-well samples (1.52- to 10.67-m wells), the other for far-well samples (45.72- to 67.05-m and control wells). The relative efficiencies of both units were tested before initiation of the sampling program, using poliovirus-seeded groundwater collected from various study wells. Recovery efficiencies for both units ranged between 65 and 87% (data not shown).

Viruses were enumerated on monolayers of Buffalo green monkey kidney cells which had been propagated in Eagle minimum essential medium with Earle balanced salt solution supplemented with 5% calf serum, glutamine, and antibiotics (penicillin, streptomycin, and gentamicin). Sample concentrates were diluted

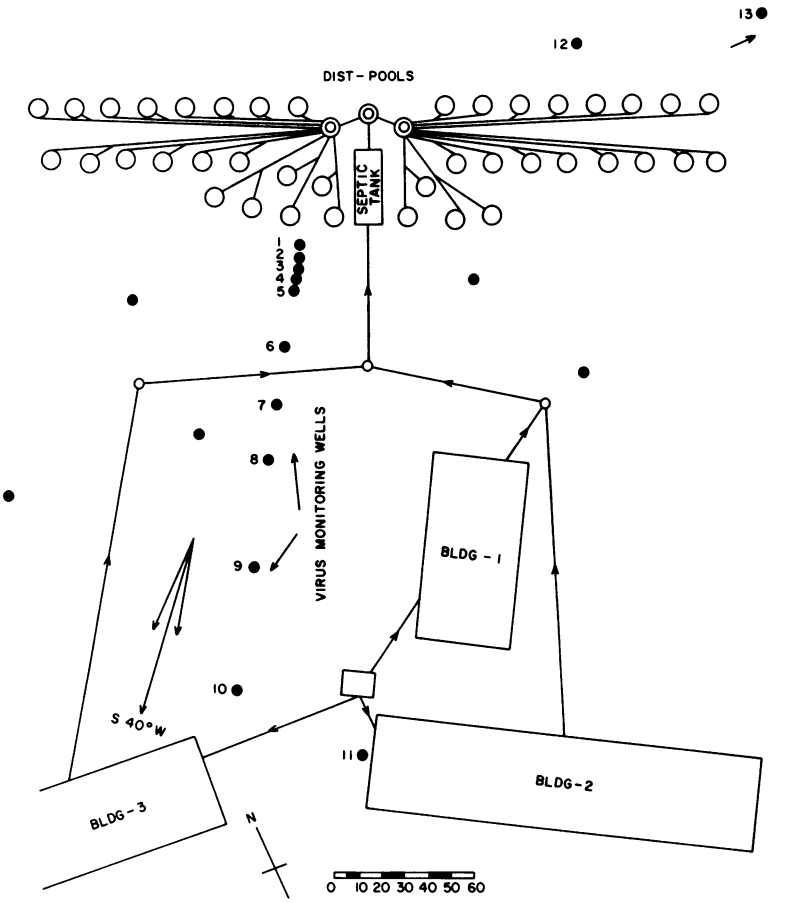

FIG. 1. Virus-monitoring well locations. Each well is numbered, and its distance from the septage source was as follows: well 1, 1.52 m; well 2, 3.05 m; well 3, 4.57 m; well 4, 7.62 m; well 5, 10.67 m; well 6, 15.24 m; well 7, 22.86 m; well 8, 30.48 m; well 9, 45.72 m; well 10, 60.35 m; well 11, 67.05 m; well 12 (north control well), 5 m up-gradient; well 13 (northeast control well), 34.6 m up-gradient. Unlabeled wells were used for determining the direction of groundwater flow and were not sampled during the present study. Well 12 was abandoned early in the study and well 13 was substituted.

(1:3), inoculated (4 ml) on Buffalo green monkey kidney cell monolayers in 75-cm^2 flasks, and incubated with rocking for 2 h to facilitate virus adsorption. After decanting excess inoculum, monolayers were overlaid with 15 ml of minimal essential plaquing medium supplemented with 2% calf serum, agar, and antibiotics (20, 21). The flasks were incubated for 1 to 3 days at 36°C under 5% CO_2 and then stained with a second overlay (10 ml) containing agar and neutral red. The flasks were then returned to the incubator and observed over an 8-day period for plaque development. All PFU were confirmed by two passages on cell monolayers. Only samples showing consistent cytopathic effect were considered positive. Because of the relatively low virus numbers expected, entire sample concentrates were assayed.

Additional measurements. Total and fecal coliform enumerations involved the use of a three-tube, most-probable-number method performed in accordance with standard methods (2). Ambient sample pH was measured with a portable pH meter (Hach model 1975). Sample conductivities were measured with a portable salinity-conductivity-temperature meter (model 33; YSI Corp.). Data were statistically analyzed by methods described previously by Sokal and Rohlf (17) and Steel and Torrie (18). Statistical analyses were performed on a Hewlett-Packard HP9845B desk-top computer, using preprogrammed statistical software.

RESULTS

Virus occurrence in septage distribution pools and shallow (1.82-m) groundwater observation wells. The system described above provided a unique opportunity for studying the characteristics of in situ virus entrainment through an aquifer receiving a constant input of domestic septic leachate. A summary of the virus recovery data from the distribution pool, as well as the control and down-gradient observation wells

screened at water table depths of 1.82 m, is presented in Table 1. Although isolates were not specifically identified, the concentration-reconcentration methods and host cell system used considerably favored the recovery of enteroviruses (21). The proximity of the test wells to a domestic wastewater disposal system left little doubt as to the human origin of the isolates. Initial samplings conducted at the control well located some 5 m to the north of the leaching pool area (well number 12) yielded virus isolated in two of five samples tested. As there was no likely up-gradient source of viruses, it was concluded that the isolations were the result of mounding of septage-contaminated groundwater occurring beneath the leaching pools. With the control value of this well compromised, sampling was transferred to a new up-gradient well, located 34.6 m to the northeast of the main leaching pool area (well number 13). All subsequent testing of this well failed to yield any virus isolates, a fact which tended to support the contention that any viruses isolated from test wells located below the leaching system (down-gradient) had originated from the system, rather than from some unknown up-gradient source.

Analysis of samples collected from the central distribution pools, which were often highly toxic to cell cultures, indicated the routine occurrence of viruses. Virus concentrations recovered from these samples (ranging from 0.07 to 148 PFU/liter) were of little predictive value, as virus residence times in distribution and leaching pools could not be determined. It was, therefore, impossible to quantitate either the total number of viruses being discharged to the leaching pools or the level being released by the leaching pools to the unsaturated soil. Qualitatively, however, the data served to indicate the regular discharge of viruses into the leaching pools.

The data from analysis of samples collected over a 12-month period from shallow, down-gradient wells indicated extensive entrainment of indigenous viruses through the aquifer (Table 1). Near-well samples (i.e., those at lateral distances of 1.52, 3.05, and 4.57 m) were frequently too toxic for tissue culture assay. Toxicity was rarely noted in samples collected from beyond the 10.67-m well, a fact which strongly suggested that the leaching pools were the principal source of near-well toxicity. Virus isolates were recovered from each of the down-gradient wells on at least one occasion. In general, virus concentrations varied with well distance from the septage source, with levels ranging from 0.002 to 10.8 PFU/liter detected in near-well samples and concentrations of 0.002 to 0.05 PFU/liter occurring in the most distant (i.e., 45.72- and 60.35-m) wells. When the data were subjected to a weighted regression analysis technique (17, 18), a significant negative correlation ($P < 0.05$; $r = -0.6492$) was revealed between the frequency of virus occurrence and increasing distance from the septage source. The weighted analytical method was used to compensate for the relatively low sampling frequency at some of the wells (i.e., numbers 3, 5, and 9), giving more credence to data from wells which had been sampled more frequently.

Coliform organisms, although occurring in high concentrations in distribution pool samples

TABLE 1. Virus recoveries from shallow-screened (1.82-m) test wells

Observation well[a]	Distance from source (m)	No. of samples collected	No. tested[b]	No. positive	Percent positive[c]
Septage distribution pool	0	15	8	7	87.5
1	1.52	28	19	8	42.1
2	3.05	16	14	5	35.7
3	4.57	3	2	1	50.0
4	6.09	16	14	5	35.7
5	10.67	10	7	3	42.8
6	15.24	16	16	1	6.2
7	22.86	26	25	4	16.0
8	30.48	22	22	4	18.2
9	45.72	3	3	1	33.3
10	60.35	22	22	2	9.1
12[d]	5.0	5	5	2	40.0
13[e]	34.6	12	12	0	0.0

[a] Data from sample well number 11, screened at an 18-m depth, appear in Table 2.
[b] Sample concentrates which were not toxic to cell cultures.
[c] (Number positive/number tested) × 100.
[d] Control well located north of the septage source.
[e] Control well located northeast of the septage source.

(10^5 to 10^8/100 ml), were rarely detected beyond the 1.52-m sample well (data not shown).

Virus occurrence in deep groundwater observation wells. By the end of the first 6 months of sampling, it had become apparent that viruses could be extensively entrained through the upper (1.82-m) portion of the aquifer. It could not be determined on the basis of the data, however, whether viruses could be carried to greater aquifer depths. The delineation of such a capacity was important, as most of the private drinking-water wells in the region draw from considerably deeper portions of the glacial aquifer. As the only deep-screened well being tested at the time was the apartment drinking-water supply (well number 11), located 67.05 m from the septage source and screened at approximately 18 m, we decided that additional wells should be installed and tested. The new wells were located in close proximity to existing test wells. One well (number 14) having a 6.09-m screen depth was sunk adjacent to the 1.52-m test well (well number 1). Three additional wells installed near the 30.48-m test well (well number 8) had screen depths of 6.09, 12.19, and 18.29 m, respectively (wells 15, 16, and 17). Sample collections, processing, and assay were performed as described above. To accommodate the influx of new samples, we discontinued testing at wells 2, 4, 5, and 6.

Attempts to recover viruses from well number 14 were unsuccessful (Table 2). This was presumably due in part to the high incidence of sample toxicity (45.5%). Isolates were recovered, however, from wells 15 and 16 (screened at 6.09- and 12.19-m depths, respectively), located some 30.48 m below the septage source (i.e., down-gradient). The presence of viruses in these wells indicated the likely mixing of septage-contaminated waters at these depths. This mixing was attributed to draw-down effects caused by sampling at the respective well points. Similar effects might be expected during normal usage of any supply well. Although viruses were not recovered from the deepest well at 30.48-m distance (well number 17), they were isolated on one occasion from the drinking-water well (well number 11), which was screened at a similar depth (18 m). Virus recovery at this latter well represented the most extensive lateral virus movement observed during the study (67.05 m).

Virus occurrence related to other measurements. Virus occurrence was compared with pH and conductivity data via product-moment correlation matrices. The analysis indicated no general relationship between any of the parameters (Table 3), although several isolated instances were noted in which a significant positive correlation occurred between pH ($\geq$6.0) and virus occurrence (e.g., wells 3, 10, and 15). These results differed dramatically from the majority of observation well samples, and no definitive relationship could be established. Likewise, a single negative correlation was observed between conductivity values and virus occurrence in well number 3 (Table 3). Because this relationship was based on only two observations, the correlation was considered to be an isolated and random event

Product-moment correlation analysis of the occurrence of total or fecal coliforms with the presence of enteroviruses in groundwater samples indicated that no significant relationship existed between these parameters (Table 4). These findings were not unexpected in light of earlier studies which had empirically demonstrated that no correlation existed between virus and coliform occurrence in sewage-impacted groundwaters on Long Island (21). The present findings provided an important statistical verification of earlier data.

Virus occurrence in groundwater as related to season. Of 280 samples collected during the course of the study, 218 were sufficiently nontoxic to permit testing on the cell culture system. Eighty-eight of these were from two spring (March through May) collections, whereas 29, 50, and 51 were from a single summer (June through August), fall (September through November), and winter (December through February) collection, respectively. Data were analyzed for seasonal fluctuations by using Friedman's test for two-way classification (18), which ranked the frequency of virus occurrence

TABLE 2. Virus recoveries from deep-screened test wells

Observation well	Distance from source (m)	Screen depth (m)	No. of samples collected	No. tested[a]	No. positive	Percent positive[b]
14	1.52	6.09	11	6	0	0.0
15	30.48	6.09	11	11	1	9.1
16	30.48	12.19	11	11	2	18.2
17	30.48	18.29	10	10	0	0.0
11	67.05	~18	13	13	1	7.7

[a] Sample concentrates which were not toxic to cell cultures.
[b] (Number positive/number tested) × 100.

TABLE 3. Product-moment correlation matrix defining the relationship between virus occurrence in observation wells and sample pH or conductivity

Observation well	Correlation coefficients[a]	
	pH	Conductivity
1	−0.2727 (17)	−0.1882 (14)
2	0.5278 (13)	0.3234 (10)
3	1.000 (2)[b]	−1.000 (2)[b]
4	−0.2680 (14)	−0.1701 (11)
5	0.6345 (7)	—[c]
6	0.0434 (16)	−0.0269 (14)
7	−0.1440 (25)	−0.0565 (22)
8	−0.0367 (23)	0.2533 (19)
9	—	0.9007 (3)
10	0.6338 (22)[b]	−0.1088 (20)
11	0.2356 (13)	−0.0308 (10)
12	−0.5708 (5)	−0.6074 (4)
13	—	—
14	—	—
15	0.6857 (11)[b]	−0.5418 (11)
16	0.3414 (11)	0.0226 (11)
17	—	—

[a] Values in parentheses represent number of observations.

[b] Significant linear correlation at $P < 0.05$.

[c] Coefficients not calculated owing to inability to recover virus at indicated sample wells.

TABLE 4. Product-moment correlation matrix defining the relationship between virus occurrence and the presence of total or fecal coliform organisms

Observation well	Correlation coefficients[a]	
	Total coliforms	Fecal coliforms
1	−0.0812 (17)	−0.0911 (15)
2	−0.0472 (13)	NF[b]
3	NT[c]	NF
4	−0.0862 (14)	−0.0263 (13)
5	0.1208 (7)	−0.1196 (7)
6	−0.0706 (16)	NF
7	−0.0448 (25)	NF
8	−0.0181 (24)	NF
9	0.5000 (3)	NF
10	−0.0808 (21)	NF
11	−0.1280 (12)	NF
12	0.6053 (5)	NF
13	NT	NF
14	NV[d]	NF, NV
15	NT	NF
16	NT	NF
17	NT, NV	NF, NV

[a] Values in parentheses represent number of observations.

[b] NF, Coefficient not calculated owing to inability to recover fecal coliforms at indicated sample wells.

[c] NT, Coefficient not calculated owing to inability to recover total coliforms at indicated wells.

[d] NV, Coefficient not calculated owing to inability to recover viruses at indicated wells.

(i.e., total number of positive samples divided by the total number tested) at each well for each season. The rankings were then summed, and a chi-square value was calculated. The test was applied only to data from down-gradient, shallow (1.82-m) wells which had been sampled during all four seasons of the year. The analysis revealed no significant difference in overall virus occurrence frequency as a function of season. This result indicated that the likelihood of recovering virus from any point along the test gradient could not be confined to a particular time of the year and that viruses appeared to be homogeneously dispersed throughout that portion of the aquifer under study.

DISCUSSION

Microbial contamination of groundwater systems represents a major source mechanism for waterborne disease outbreaks (4, 5, 13, 14). In most cases, contamination of shallow aquifers has been the direct result of wastewater disposal at or near the soil surface, with subsequent lateral movement through the aquifer (1). The most often identified sources of contamination have been subsurface septic systems and cesspools (3).

The most commonly identified agent in drinking water-associated outbreaks occurring between 1971 and 1977 was *Giardia lamblia* (5). However, 57% of the recorded outbreaks could not be associated with a specific etiological agent, a fact which led Craun (5) to speculate on the possibility of a virus etiology. The potential for waterborne virus disease transmission underscores the need for identifying the fate of viruses in a sole-source aquifer.

In the present study, viruses were isolated from groundwater at distances of up to 67 m down-gradient from the leaching pools of a subsurface wastewater disposal system. Although a majority of isolates were recovered from wells drawing from the upper portion of the aquifer, the potential for extensive vertical entrainment of viruses within the aquifer was evidenced by virus recovery from wells which had been screened at 6-, 12-, and 18-m depths. The specific limits of virus entrainment through the system could not be determined, as wells had not been installed beyond 67 m (down-gradient) or deeper than 18 m.

Current building codes in many portions of the county where groundwater serves as both a potable water source and an eventual recipient of domestic wastewater require that a minimum distance of 30.48 m be observed in the placement of domestic subsurface disposal systems and down-gradient private drinking water wells. In those instances in which the minimum distance cannot be applied, a supplementary regulation requires that wells be installed an addi-

tional 0.91 m in depth for each 0.3 m under the recommended minimum distance. The results of the present study indicate the prudence of questioning this regulation in areas having a hydrogeological profile similar to that of Long Island. In such regions, should virus-free water be desired, it may be advisable to consider either increasing the lateral placement distance or drilling significantly deeper wells. The virological ramifications of such measures are currently being pursued by this laboratory and by the Suffolk County Department of Health Services.

Statistical analysis indicated no overall patterns of significant correlation between virus occurrence and occurrence of fecal and total coliform organisms. This result, in conjunction with similar findings from previous studies (3, 15, 16), provided strong evidence repudiating the use of current microbial water quality standards for predicting the likely virological quality of groundwater.

ACKNOWLEDGMENTS

This project was conducted by Brookhaven National Laboratory under a contract with the Suffolk County Department of Health Services, N.Y. We wish to acknowledge the invaluable efforts of Aldo Andreoli, Joseph Baier, and Craig Wherle, Suffolk County Department of Health Services, and Lois Baranosky, Marilyn Dahl, and Cheryl Beckwith, Brookhaven National Laboratory, in the preparation and conduct of this research. Statistical analyses were kindly provided by Keith Thompson, biostatistician, Brookhaven National Laboratory.

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