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Human Pathogenic Viruses at Sewage Sludge Disposal Sites in the Middle Atlantic Region

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Human enteric viruses were detected in samples of water, crabs, and bottom sediments obtained from two sewage sludge disposal sites in the Atlantic Ocean. Viruses were isolated from sediments 17 months after the cessation of sludge dumping. These findings indicate that, under natural conditions, viruses can survive for a long period of time in the marine environment and that they may present potential public health problems to humans using these resources for food and recreation. The isolation of viruses in the absence of fecal indicator bacteria reinforces previous observations on the inadequacy of these bacteria for predicting the virological quality of water and shellfish.

Human enteric viruses have repeatedly been shown to be present in domestic wastewater, often in large numbers. As many as 110 different virus types may be present in sewage and may not be completely removed by conventional sewage treatment processes, including chlorination (23). In an activated-sludge sewage treatment plant, removal of viruses occurs by adsorption of viruses to solids, which ultimately settle out in the form of sludge flocs. Thus, the concentration of viruses in sludge may be several orders of magnitude higher than in sewage or effluent or both, and the discharge of sludge in coastal waters is, therefore, potentially hazardous to human health. In fact, several outbreaks of infectious hepatitis and viral gastroenteritis have been traced to sewage contamination of recreational water and shellfish (3, 7, 14).

Coastal marine waters are a valuable recreation and food resource but have historically been used as a convenient receptacle for human waste, either by ocean outfalls or barge disposal. Because of the population explosion and industrial growth, these areas are now increasingly susceptible to pollution. Enormous amounts of sewage sludge generated by densely populated coastal towns are often barged several miles away from the coast and discharged in deep midshelf waters. A large amount of sewage sludge has been dumped at various designated sites around the world. The present study concerns the occurrence and survival of human enteric viruses at two sewage sludge disposal sites in the Atlantic Ocean.

MATERIALS AND METHODS

Dump sites. Two sewage sludge disposal sites located in the Atlantic Ocean were studied. (i) The New York Bight dump site (NYB), also known as the 12-mi. (19.2-km) site, is a coastal oceanic area at the apex of New Jersey and Long Island; it is located at 40°25′04″ N, 73°44′53″ W. The sludge dumping area is ca. 30 m deep and occupies 100 km^2. Sewage wastes are disposed of at this dumping ground by several cities in New York and New Jersey and are either in a raw or treated state or in a digested form. An estimated 3.5×10^6 tons (3.15×10^6 t) of wastes are discharged every year in this area (27). (ii) The Philadelphia sewage sludge dump site (PDS) is a 172-km^2 area located 70 km east of Ocean City, Md., at ca. 38°23′ N, 74°15′ W, it lies over the continental shelf in waters 40 to 60 m deep. Sewage sludge from Philadelphia, Pa., and Camden, N.J., was dumped at this site from 1973 through 1980. The site received ca. 305×10^6 kg of sludge before dumping ceased on 25 November 1980 (26). The locations of both sites are shown in Fig. 1.

Sample collection. Between 1980 and 1982, three scientific cruises were made to PDS and two scientific cruises were made to NYB for the collection of water, sediment, and rock crabs (*Cancer irroratus*). During these cruises, 111 and 73 different stations were sampled in PDS and NYB, respectively. In addition, 29 other stations located between the two sites were sampled during the first 2 years. Bottom sediments were collected from all stations, and water and crab samples were collected from a few selected stations.

(i) Water samples. For bacteriological examination, water samples were collected with a sterile hinge sampler (25). For virological examination, water samples were processed with the aid of a virus concentrator as described below.

(ii) Sediment samples. Sediment samples were collected with a 0.1-m^3 Smith-McIntyre sampler (16). The top 1-cm layer of the sediment was removed with a sterile tongue depressor and placed in a sterile plastic bag. All sediment samples were routinely refrigerated and analyzed for fecal indicator bacteria on board within 6 h of collection. For quantitation of viruses, the samples were frozen and later shipped to a virology laboratory, where they were stored at −70°C pending virus isolation.

(iii) Rock crabs. Rock crabs (*C. irroratus*) were collected with a 3/4 Yankee trawl or a rocking chair dredge. Tows were done for 15 to 30 min at 1.5 to 3.5 knots. The gastrointestinal tracts and hepatopancreases of 10 to 12 crabs from each station were pooled, frozen, and then shipped to the laboratory for virus isolation.

Indicator bacteria. Most probable numbers of total coliforms, fecal coliforms, and fecal streptococci were determined by standard methods (2) as described previously (26).

Concentration and detection of viruses. (i) Water. Viruses from 400- to 800-liter samples of bottom seawater were concentrated by the membrane adsorption-elution method (11, 12). Each water sample was adjusted to pH 3.5 and 0.001 M $AlCl_3$ by in-line injection of 1 N HCl and salt and was pumped through a series of two virus-adsorbent filters of 3.0- and 0.45-μm porosity (Filterite filters, Duo-Fine series; Filterite Corp., Timonium, Md.). Adsorbed viruses were

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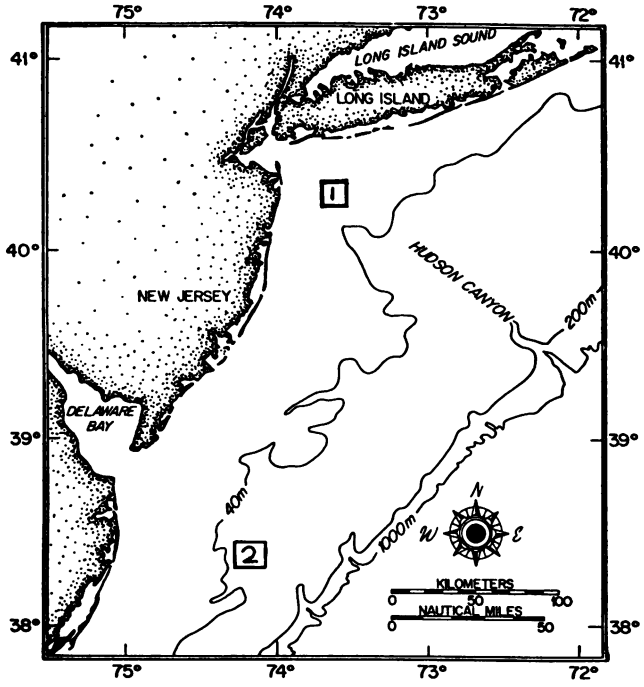

FIG. 1. Locations of NYB (1) and PDS (2).

eluted by passing 1.5 liters of 6% beef extract (Lab-Lemco; Oxoid Ltd., Hants., United Kingdom) solution (pH 10.5) through the filters. This primary eluate was further reconcentrated by a modified organic flocculation procedure (12, 18) as described below. The virus-containing eluate was acidified to pH 3.5, resulting in the formation of a virus-containing organic floc. Viruses from this floc were eluted by suspending the floc in a small volume of 0.05 M glycine buffer (pH 11.0). The floc was discarded after centrifugation, and the supernatant was adjusted to a neutral pH and assayed for viruses.

(ii) Sediment. Viruses from sediment were eluted by suspending 300 to 400 g of sediment in 5 volumes (1.5 to 2 liters) of 6% beef extract solution (pH 10.5), shaking the mixture vigorously for 5 min, and removing the sediment by low-speed centrifugation. The virus-containing supernatant was reduced in volume by the modified organic flocculation method as described above.

(iii) Crabs. Viruses from pools of crab tissues were extracted in 7 volumes of 0.05 M glycine buffer (final pH, 9.2). The volume of this virus-containing extract was reduced by a concentration step involving acid precipitation (29). Briefly, the extract was adjusted to pH 3.5, the supernatant was discarded after centrifugation, and the pellet was suspended in a small volume of glycine-saline (pH 10.5). Cat-Floc (Calgon, Pittsburgh, Pa.) was added at a final concentration of 5%, and the mixture was centrifuged at 2,000 × *g* for 20 min. The supernatant was neutralized, treated with antibiotics, and assayed for viruses (29).

Isolation and identification of viruses. All samples were assayed on BGMK (buffalo green monkey kidney) cells by plaque assay and examination of cytopathic effects as previously described (13). Virus isolates were identified by virus neutralization tests with Lim-Benyesh-Melnick antiserum pools and specific antisera (24).

Detection of rotaviruses. All samples were tested for the presence of human rotaviruses by an indirect immunofluorescence assay as described by Smith and Gerba (30).

RESULTS

PDS. As shown in Table 1, 22 water, 111 sediment, and 11 crab samples were obtained from this site during the 3 years of the study. The locations of the stations sampled and the stations yielding human enteroviruses are shown in Fig. 2. None of the water samples at PDS was found to contain viruses. In 1980, 1981, and 1982, sediments from two stations each yielded viruses. Of 11 crab samples collected in 1982, 2 were positive for coxsackievirus B3. Viruses were isolated from some stations at which the concentrations of indicator bacteria were below detection limits (Table 2).

NYB. In 1980, 5 of 30 sediment samples and 0 of 8 crab samples yielded human enteroviruses. In 1981, 43 sediment and 13 crab samples were collected. Of these, seven sediment samples and one crab sample yielded viruses. The viruses isolated are shown in Table 3, and the station locations are shown in Fig. 3. Water samples were not collected from this site.

Transect between the two dump sites. The isolation of viruses from stations located between the two dump sites is shown in Table 4. In 1980, both water and sediment samples from one station (KN49) yielded viruses. Sediments from two other stations (80-6 and SH45) were also positive for viruses in 1980. In 1981, sediments from six different stations yielded viruses. It is interesting to note that sediments from station 80-6 yielded viruses in both 1980 and 1981 but that the virus types isolated were different. Also, sediments from station 80-6 in 1981 were contaminated with both echovirus 1 and poliovirus 2. Viruses often were isolated when indicator bacteria were below detection limits. Human rotaviruses were not detected at any of the sites.

DISCUSSION

PDS is located 40 mi. (64 km) offshore. Viruses isolated from this site probably originated from sludge barges rather than from other anthropogenic sources. Although dumping

TABLE 1. Number of samples obtained from PDS, NYB, and an area between the two sites between 1980 and 1982

Yr	No. of samples obtained (no. yielding viruses)[a]						
	PDS			NYB		Transect between the two sites	
	Water	Sediment	Crabs	Sediment	Crabs	Water	Sediment
1980	5 (0)	34 (2)	—[b]	30 (5)	8 (0)	5 (1)	8 (3)
1981	9 (0)	41 (2)	—	43 (7)	13 (1)	9 (0)	21 (5)
1982	8 (0)	36 (2)	11 (2)	—	—	—	—

[a] Samples of water (400 to 800 liters), bottom sediments (300 to 400 g), and pools of crab tissues (25 to 40 g) were examined for the presence of human enteric viruses.

[b] —, Not done.

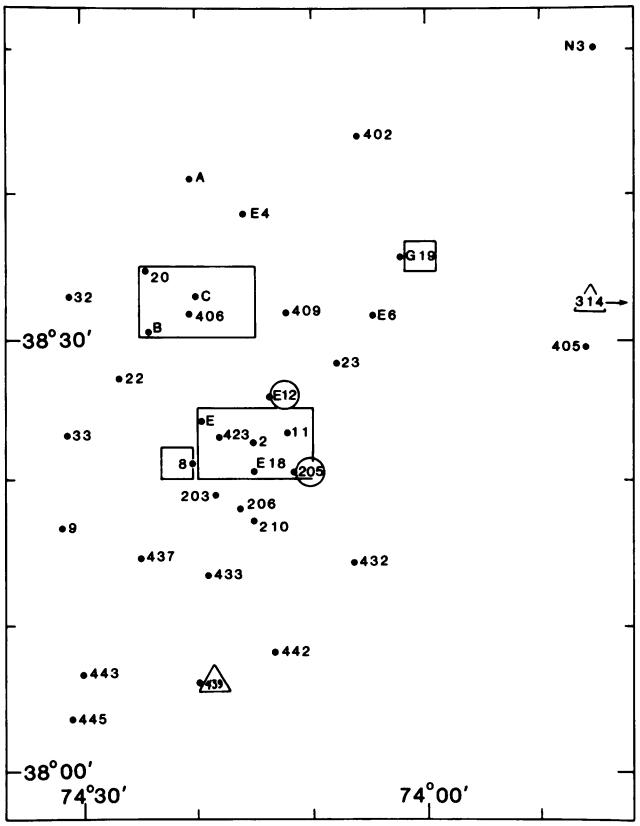

FIG. 2. Stations at PDS positive for viruses in 1980 (□), 1981 (○), and 1982 (△). The lower rectangle represents PDS. The upper rectangle outlines an inactive acid waste dump site.

ceased in November 1980, viruses were still isolated in May 1981 and June 1982, indicating that the viruses survived for at least 17 months under existing field conditions. This finding is supported by the results of several laboratory studies in which viruses were found to survive for a long time in estuarine and marine environments (1, 21). Coxsackievirus B3 was the only virus isolated in 1982, indicating that this virus may survive longer than other enteroviruses (22).

It should be pointed out that all samples were tested for the presence of viruses by plaque assay under an agar overlay and by cytopathology under a liquid medium. Most of the samples which yielded viruses did so in both procedures. However, a few samples were positive as determined by the examination of cytopathic effects only and did not produce plaques. We have observed this phenomenon on several different occasions in our laboratory (S. M. Goyal, unpublished data), and we suggest that all environmental samples be examined by both plaque assay and cytopathology. It is also worth mentioning that some of the samples (including the crab samples) did not yield viruses on the first passage and yielded viruses only when passaged two or three times.

Most of the stations positive for viruses were located between 40- and 70-m isobaths in and around the disposal site (Fig. 2), but, unexpectedly, some midshelf areas were found to be contaminated. Thus, the isolation of viruses from crabs at station 314 was unexpected because this station lies well to the east of the dump site. Sediments from this station also contained pathogenic *Acanthamoeba* spp. (T. K. Sawyer, personal communication), indicating that sludge from the disposal site or other sources may have dispersed over long distances, probably because of wind-induced currents and geostrophic flow.

Stations 5, 7, and 9 are located very close to NYB (Table 3, and Fig. 3). It is reasonable to assume, therefore, that the

TABLE 3. Human enteric viruses isolated from NYB

Yr	Station	Depth (m)	Sample	Virus[a]	No. of viruses (PFU)[b]
1980	5	35	Sediment	U	18
	20	12	Sediment	E1	64
	21	21	Sediment	U	60
	30	34	Sediment	E7	182
	34	54	Sediment	E1	56
1981	4	20	Sediment	CB3	108
	7	25	Sediment	CB5	12
	9	36	Sediment	E1	30
	14	74	Sediment	E1	4
	19	14	Sediment	CB3	84
	25	19	Sediment	CB3	14
	39	42	Sediment	E1	2
	62	23	Crabs	CB3	CPE[c]

[a] See Table 2, footnote *a*, for some definitions. E7, Echovirus 7; CB5, coxsackievirus B5.
[b] See Table 2, footnote *b*.
[c] See Table 2, footnote *e*.

TABLE 2. Isolation of viruses from PDS and their relationship to fecal indicator bacteria

Yr	Station	Depth (m)	Sample	Virus[a]	No. of viruses (PFU)[b]	Indicator bacteria per 100 ml of [c]:					
						Water			Sediment		
						TC	FC	FS	TC	FC	FS
1980	8	46	Sediment	E1	20	—[d]	—	—	60	60	<36
	G19	65	Sediment	U	46	—	—	—	<46	—	<36
1981	E-12	49	Sediment	P2	12	—	—	—	<46	<46	60
	205	57	Sediment	E9	8	—	—	—	<46	<46	<46
1982	E-4	41	Sediment	CB3	CPE[e]	2	<1	<1	<22	<22	<22
	439	42	Sediment	CB3	7	<1	<1	<1	<22	<22	<22
	314	77	Crabs	CB3	12	<1	<1	<1	<22	<22	<22
	409	58	Crabs	CB3	3	<1	<1	<1	<22	<22	<22

[a] E1, Echovirus 1; U, unidentified virus; P2, poliovirus 2; E9, echovirus 9; CB3, coxsackievirus B3.
[b] PFU of virus per kilogram of sediment or 100 g of crab pools.
[c] TC, Total coliforms; FC, fecal coliforms; FS, fecal streptococci.
[d] —, Not done.
[e] The virus was isolated only by cytopathology under a liquid medium and not by the plaquing procedures.

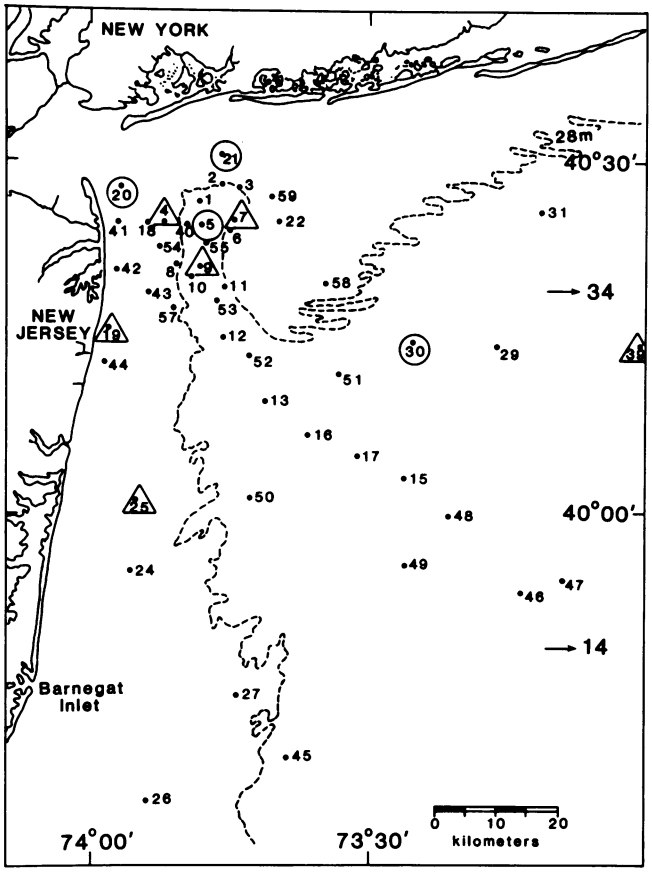

FIG. 3. Stations at NYB positive for viruses in 1980 (○) and 1981 (△).

source of viruses isolated from these stations was dumped sludge. Stations 14, 34, and 39, however, are located on the midshelf off eastern Long Island. It is difficult to determine if the input of viruses at these stations was the Hudson Plume or sludge dumping. It should also be pointed out that crabs from station 35, which is not very far from station 34, yielded both total and fecal coliforms. The isolation of pathogenic viruses from sediments, outside the shellfish closure area raises serious questions about potential hazards to humans using the Bight as a food and recreation resource.

The isolation of coxsackievirus B5 from sediments at station SH45 was of interest (Table 4) because this station is located only 1.6 nautical mi (3.0 km) off the summertime resort beaches of Asbury Park and Ocean Grove, N.J., and we can recall seeing summer beach activities from the ship at the time of sampling. The presence of viruses at this station raises the question of whether the contamination is (i) from local sources, (ii) part of the material from NYB ca. 15 nautical mi. (27.8 km) northeast of this station, (iii) from the Hudson River-Raritan River system 20 nautical mi. (37.0 km) north of this station, or (iv) from all three. Station KN49, located ca. 8.4 nautical mi. (15.6 km) southeast of the sludge release site, was also unique because both water and sediment samples yielded viruses. As the amount of dumped sludge appears to be miniscule in relation to the volume of receiving waters, the isolation of viruses from water, although only occurring once during the study, appears to be significant.

Of a total of 213 sediment samples examined during this study, 26 (12%) were positive for enteroviruses, whereas only 1 of 36 water samples yielded viruses. This is not surprising, as most of the discharged sludge quickly settles to the bottom of the sea and forms a part of the bottom sediments (17), which play a major role in the distribution, survival, and transport of bacteria and viruses in the marine environment (20). It has been shown that viruses are present in sediments at a much higher concentration than in overlying water and that viruses associated with sediments survive longer than those that are free in suspension (9, 19). Also, viruses associated with solids are as infectious to animals and cell cultures as those that are freely suspended (28). These observations have led some investigators to suggest that sediments may act as long-term reservoirs of viruses and that the latter may be released into the water column upon resuspension of sediments, creating a potential public health problem (9, 20). It has also been shown that water temperature plays a significant role in the survival of viruses in water (21). Low temperatures have been found to prolong virus survival, whereas higher temperatures inactivate viruses (21). In the present study, a protective sludge-sediment matrix and a generally low bottom temperature (ca. 7°C) may have contributed to the prolonged virus survival.

To our knowledge, this is the first reported isolation of human pathogenic viruses from edible crabs. Several outbreaks of infectious hepatitis and viral gastroenteritis have been traced to the consumption of shellfish harvested from

TABLE 4. Viruses isolated from stations located between the two dump sites

Yr	Station	Depth (m)	Sample	Virus[a]	No. of viruses (PFU)[b]	Indicator bacteria per 100 ml of[c]:				
						Water		Sediment		
						TC	FC	TC	FC	FS
1980	80-6[d]	27	Sediment	CB3	50	<1	<1	60	<46	270
	SH45	18	Sediment	CB5	15	8	<1	620	230	2,400
	KN49	58	Sediment	CB5	12	3	—[e]	<46	—	36
	KN49	58	Water	CB3	2	3	—	<46	—	36
1981	80-6[d]	27	Sediment	E1, P2	22	<1	<1	<46	<46	<46
	81-11	21	Sediment	P2	30	10	7	60	<46	<46
	81-14	21	Sediment	CB5	8	<1	<1	130	<46	60
	KN46	42	Sediment	E1	56	—	—	<46	<46	<46
	KN50	24	Sediment	E1	4	≥300	—	2,400	60	—

[a] See Table 2, footnote *a*, and Table 3, footnote *a*, for definitions.
[b] PFU of virus per kilogram of sediment or 400 liters of water.
[c] See Table 2, footnote *c*.
[d] Sediments from station 80-6 yielded viruses in both 1980 and 1981.
[e] —, Not done.

sewage-polluted waters (7, 14). Shellfish beds are closed to harvesting if evidence of fecal pollution of these waters exists. The closure of shellfishing, however, applies only to the commerical harvesting of mollusks such as surf clams and ocean quahogs and does not apply to recreational fishing. It also does not apply to the commercial harvesting of nonmolluscan shellfish, such as crabs and lobsters, which are also known to accumulate viruses and bacteria from water, sediments, or food. Because these accumulated microorganisms generally do not concentrate in the edible tissues of these animals, they have not been extensively studied. Nevertheless, the potential health effects of such accumulations should not be dismissed, because traditional cooking methods may not be adequate for inactivating all the accumulated pathogens (6, 15).

The sanitary quality of recreational water, edible shellfish, and shellfish-harvesting waters is based on the levels of coliforms and fecal coliforms as indicators of fecal contamination and on the identification of sources of fecal contamination through sanitation surveys. Recent epidemiological and microbiological findings, however, have raised serious concerns about the adequacy of fecal indicator bacteria for predicting the virological quality of water and shellfish (4, 8, 10). In the present study, no correlation was detected between the presence of viruses and of indicator bacteria at both dump sites. Thus, viruses were isolated from several sediment samples when total and fecal coliforms were absent or were below detection limits (Tables 2 and 4). It is interesting to note, however, that several stations (e.g., E12, 80-6, 205, 314, KN46, KN50, and 409) from which viruses were isolated also yielded pathogenic *Acanthamoeba* spp. (T. K. Sawyer, personal communication). In future studies, attempts should be made to further explore this relationship.

The 1977 Amendment of the Marine Protection, Research, and Sanctuaries Act of 1972 prohibits the dumping after 31 December 1981 of any sewage sludge which would be harmful to marine ecosystems and human health. However, ocean dumping of sewage sludge still occurs and may, in fact, be increasing (31). Recent outbreaks of clam-associated hepatitis and viral gastroenteritis in the New York area (5) and the isolation of human pathogenic viruses from crabs, water, and sediments at sewage sludge dump sites point to the need for continued monitoring and surveillance of these sites so that appropriate steps can be taken to safeguard human health. These studies should also be helpful in making future management decisions for the rational and beneficial use of marine ecosystems. Continued monitoring of PDS should also be useful in determining the environmental recovery patterns that develop at this site after the cessation of dumping.

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