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Human Enteroviruses in Oysters and Their Overlying Waters

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The presence of enteroviruses in oysters and oyster-harvesting waters of the Texas Gulf coast was monitored over a period of 10 months. Viruses were detected in water and oyster samples obtained from areas both open and closed to shellfish harvesting. Viruses were detected periodically in waters that met current bacteriological standards for shellfish harvesting. No significant statistical relationship was demonstrated between virus concentration in oysters and the bacteriological and physicochemical quality of water and shellfish. Viruses in water were, however, moderately correlated with total coliforms in water and oysters and with fecal coliforms in oysters. Total coliforms in water were related to total coliforms and fecal coliforms in oysters and to fecal coliforms in water. Fecal coliforms in sediment were related only to total coliforms in sediment. Among the physicochemical characteristics of water, turbidity was related statistically to the organic matter content of water and to fecal coliforms in water. There was a marked effect of rainfall on the bacteriological quality of water. Of a total of 44 water samples, 26 yielded virus in concentrations from 4 to 167 plaque-forming units per 100-gallon (ca. 378.5-liter) sample. Of a total of 40 pools of 10 to 12 oysters each, virus was found in 14 pools at a concentration of 6 to 224 plaque-forming units per 100 g of oyster meat. On five occasions, virus was found in oysters but not in the overlying water. Similarly, viruses were isolated from 17 water samples when no virus could be detected in oysters harvested from the same sites. This study indicates that current bacteriological standards for determining the safety of shellfish and shellfish-growing waters do not reflect the occurrence of enteroviruses.

The coastal areas of any nation are a vital recreational resource and food source. This valuable resource, however, is lost when humans overload the natural self-purification system with their wastes. Viruses usually occur in domestic sewage and survive in some numbers even after secondary treatment (17). They can also survive in seawater for a few days to several weeks (32). By virtue of their ability to concentrate viruses and bacteria, shellfish such as oysters, mussels, and clams may become actively contaminated even at considerable distances from the point of sewage discharge. Because these species are often eaten raw or lightly cooked along with their gastrointestinal tract, the risk of illness after their consumption may be high.

Effective control of enteric bacterial disease spread by shellfish has resulted from the establishment of bacteriological standards by using total coliform and fecal coliform indexes as the basis for a certification program. Less documented and less studied are the problems associated with the transmission of viral disease by shellfish because of the lack of sensitive techniques for their study. Documented outbreaks of hepatitis have been attributed to the consumption of shellfish, which emphasizes both the need for definitive information on virus pollution of coastal waters and shellfish and the inadequacy of present bacteriological criteria for the sanitary quality of shellfish and shellfish-harvesting waters.

Even in nonepidemic periods, there is a relationship between the consumption of shellfish and the occurrence of hospitalized cases of infectious hepatitis (24). It would seem that the true incidence of shellfish-associated viral diseases is greatly underestimated (18). In addition, residents of coastal states seem to have a higher incidence of infectious hepatitis (6). The predominantly occult nature of other waterborne viruses has made it difficult to document their transmission by shellfish, but their occurrence in shellfish is well documented (12, 35, 42) and their potential for disease always exists.

Although a number of studies have been reported on the isolation of viruses from shellfish (2, 10, 32, 33), definitive field studies on viral contamination of coastal waters and shellfish are

limited. The presence of enteroviruses in sewage and sewage-polluted coastal waters (9, 20, 39) and the occurrence of infectious hepatitis and nonbacterial gastroenteritis outbreaks due to the ingestion of raw shellfish (5, 7) indicate the need for field studies on the presence of viruses in shellfish and their relationship to indicator bacterial organisms. The present study was undertaken to assess the nature, extent, and sanitary significance of enterovirus pollution in the waters and shellfish of the Texas Gulf coast. These findings were then compared statistically with various physicochemical characteristics and bacteriological quality of the water.

MATERIALS AND METHODS

Sampling sites. Samples were obtained from Galveston Bay in four areas that were approved for shellfish harvesting as well as from six areas closed to harvesting. The names of the study sites, their status as to shellfish harvesting, and the physicochemical characteristics of water samples from these sites are given in Table 1.

Indicator organisms. Most probable numbers (MPN) of total coliforms were determined by using a four-dilution, five-tube replication of lactose broth (Baltimore Biological Laboratory, Cockeysville, Md.). Later in the study, this was changed to a four-dilution, three-tube replication series. Sample volumes of 10.0, 1.0, 0.1, and 0.01 ml of surface water were inoculated into the appropriate tubes. For sediment and oyster samples, appropriate 10-fold dilutions yielding final sample volumes of 1.0, 0.1, 0.01, and 0.001 ml were used. Oyster meat was homogenized for 30 s before bacteriological examination. The lactose broth tubes were incubated at 35°C for 48 h, and tubes showing the production of acid and gas were recorded as presumptive for total coliforms.

Positive tubes of lactose broth were used to inoculate EC broth (Baltimore Biological Laboratory) tubes that were then incubated at 44.5°C. Tubes showing the production of acid and gas after 24 h were recorded as confirmed for the presence of fecal coliforms. The MPN indexes for total and fecal coliforms were computed by using published tables (1).

Salmonellae. Initially, MPN of salmonellae were determined by using a three-dilution, three-replication series of tetrathionate broth tubes (Baltimore Biological Laboratory). Because no salmonella was isolated by this method in several examinations, it was later changed to a qualitative method in which surface water, sediment samples, and oyster meat homogenate were inoculated into duplicate flasks of double-strength tetrathionate broth. The sample volumes used were 100 ml, 100 g, and 100 g, respectively. After incubation for 48 h at 41.5°C (19), a loopful of culture was streaked on brilliant green sulfonamide plates that were incubated at 37°C for 24 h. Salmonella-like colonies were picked, purified, and inoculated into triple sugar iron agar slants. Suspected cultures were tested against salmonella polyvalent antiserum (Baltimore Biological Laboratory) in a slide agglutination test. No salmonella, however, was isolated from any of the samples.

Oysters. Oysters were washed and scrubbed thoroughly in running tapwater, treated with 70% alcohol, and opened aseptically. Meat from 10 to 12 oysters was mixed, blended in a Waring blender for 30 s, and

TABLE 1. *Physicochemical characteristics of water at collecting stations*

Station	Oyster harvesting status	No. of samples tested	pH	Temp (°C)	Salinity (ppt)[a]	Turbidity (NTU)[b]	Organics (OD at 0.254 nm)[c]
April Fool's Point	Closed	9	8.2–8.8 (8.3)[d]	24–35 (30)	10–22 (17.2)	9–50 (25.3)	0.203–0.354 (0.270)
Mowle's Bait Camp	Closed	6	8.0–8.8 (8.3)	27–35 (31)	8–18 (14.7)	18–85 (42.3)	0.350–0.520 (0.411)
Red Bluff Reef	Closed	2	8.0–8.7 (8.4)	20–31 (26)	12–12 (12)	16–59 (37.5)	0.511–0.750 (0.630)
Eagle's Point	Closed	6	8.0–8.4 (8.2)	18–31 (28)	10–21 (16.8)	12–54 (35.5)	0.166–0.491 (0.335)
Tiki Island	Closed	6	7.8–8.2 (8.0)	21–32 (28.5)	18–32 (25.3)	9–43 (23.8)	0.159–0.548 (0.314)
Yacht Club	Closed	1	8.7	32	13	8	0.260
North Redfish	Approved	3	8.3–9.0 (8.7)	12–29 (18)	16–18 (16.7)	9–14 (11.7)	0.196–0.316 (0.275)
South Redfish	Approved	4	8.1–8.5 (8.3)	11–30 (20.5)	12–18 (15.5)	12–16 (13.8)	0.230–0.320 (0.273)
Reef 59	Approved	4	8.3–8.5 (8.4)	30–32 (31)	13–18 (15.8)	5–13 (8.2)	0.190–0.248 (0.219)
Reef 63	Approved	3	8.4–8.5 (8.5)	31–31 (31)	13–17 (14.3)	6–9 (7.3)	0.200–0.246 (0.226)

[a] ppt, Parts per thousand (grams per liter).
[b] NTU, Nephalometric turbidity units.
[c] OD, Optical density.
[d] Numbers in parentheses represent arithmetic means.

used for bacteriological and virological examinations.

Detection of viruses in oysters. A modification of the method devised by Sobsey et al. (40) was used for virus detection in oysters. Pooled oyster meat was blended with four times its volume of 0.05 M glycine buffer, pH 9, for 10 s. This homogenate was centrifuged at 2,000 × *g* for 10 min after adjusting the pH to 9.0. The supernatant obtained was adjusted to pH 5.5 with pH 2 glycine and to a salt concentration of less than 1,500 mg of NaCl per liter. Under these conditions viruses adsorb to oyster solids (40). After recentrifugation, the supernatant was discarded and virus was eluted from the solids by using a small volume of glycine–saline at pH 11.5. The eluate was neutralized and made to contain 2% fetal calf serum. This eluate was assayed in the cell culture system after treatment with penicillin, streptomycin, and mycostatin. The overall efficiency of this method was found to be about 60%.

Concentration and detection of viruses in water. A modified Wallis-Melnick virus concentrator was used to concentrate viruses from seawater samples as detailed elsewhere (20, 37). Water samples were adjusted to pH 3.5 and 0.0015 M $AlCl_3$ by the use of an in-line injection system and then passed through the virus-adsorbent filters, which consisted of a combination of a K27 fiberglass depth filter cartridge (Commercial Filter Division, Carborundum Co., Lebanon, Ind.) and a 0.45 μm-porosity pleated filter (Duo-Fine filter series, Filterite Corp., Timonium, Md.) in series. Virus adsorbed to the filters was eluted by passing 2 liters of 0.05 M glycine buffer, pH 11.5, through the filters. The eluate was neutralized with glycine buffer, pH 2, and the volume of this eluate was further reduced to 20 to 40 ml by aluminum flocculation and hydroextraction (11). The average overall recovery efficiency of this method was approximately 50%.

Virus isolation. Virus isolation and assays were performed by the plaque-forming unit (PFU) method or by the cytopathogenic method as detailed elsewhere (13). The BGM monkey cell line (8) was used for detection of viruses. The cells were passaged, grown, and maintained as previously described (29). A selected number of plaques were plucked and identified by pools of enterovirus antisera (28). The results are reported as PFU per 100 gallons (ca. 387.5 liter) of water or 100 g of oyster meat or sediment. Depending upon the virus concentration, 50 to 100% of the total eluate volume of each sample was assayed. Some of the poliovirus isolates were subjected to the delayed marker test (22) and the temperature marker test (3) for determining properties associated with virulence.

Physicochemical parameters of water samples. Salinity, turbidity, pH, and temperature of water samples were determined as described previously (19). In addition, the optical density of the water samples was observed at 254 nm with a spectrophotometer. The absorbance of ultraviolet at this wavelength has been shown to be related to the soluble organic content of seawater and can be used as an indicator of pollution.

Statistics. Product-moment correlation coefficients between all the parameters, i.e., rainfall, pH, temperature, salinity, organics and turbidity of water, viruses in water and in oyster samples, and total and fecal coliforms in water and in sediment samples were calculated on a DEC 10 computer by using the SPSS (Statistical Package for the Social Sciences) statistical package (36). Significant statistics were calculated for each of the coefficients.

RESULTS

Indicator bacteria. MPN of indicator bacteria (total and fecal coliforms) were determined in samples of water, sediment, and oysters taken from the 10 sites representing "open" and "closed" areas. The number of samples taken from each site varied between one and nine during the 10-month period (Table 1). Total and fecal coliforms were widely distributed at these study sites, and there was a considerable degree of fluctuation in their numbers. The population of total coliforms was found to vary between <3 and 4,600 in water, between 430 and 240,000 in bottom sediment, and between 40 and 110,000 in oysters. The figures for fecal coliforms were between <2 and 2,400, <2 and 46,000, and <2 and 46,000 for water, sediment, and oyster samples, respectively. In general, higher numbers of both total and fecal coliforms occurred in the bottom sediments and oysters than in surface water samples (Table 2). On several occasions, oyster samples contained more organisms than did bottom sediments, whereas on other occasions the opposite was true. The sediment:water ratio was between 3 and 10,435 (median = 107) for total coliforms and between 0.5 and 3,500 (median = 10) for fecal coliforms. Only three water samples had fecal coliform numbers greater than those in sediments. Similarly, the oyster:sediment ratios were between 0.1 and 258 (median = 5) and between 0.04 and 122 (median = 1) for total and fecal coliforms, respectively. The MPN of total and fecal coliforms in oysters were higher than those in sediments on 7 (23%) and 17 (50%) occasions, respectively.

Greater numbers of bacteria were isolated after rainfall, which may be due partially to the addition by surface runoff of coliforms from nonpoint sources. The numbers of bacteria generally were higher in oysters and sediments taken from closed areas, which is not unexpected. MPN of total coliform bacteria in water samples from open areas never exceeded 43/100 ml, which is well within the range suggested for shellfish harvesting. Oysters harvested from open areas exceeded the limit for fecal coliforms only once when the MPN was 750/100 g of meat (at reef 59). However, the MPN of total coliforms in bottom sediments of open areas was as high as 15,000 and was never lower than 430. As shown in Fig. 1, the numbers of fecal coliforms in water,

TABLE 2. *Populations of indicator bacteria in water, sediment, and oysters*

Station and sample	MPN (per 100 ml) of:	
	Total coliforms	Fecal coliforms
Surface water		
April Fool's Point	23-≥ 2,400[a] (472)[b]	<2-150 (46)
Mowle's Bait Camp	23-4,600 (1,179)	7-≥ 2,400 (421)
Red Bluff Reef	23-23 (23)	4-8 (6)
Eagle's Point	460-1,100 (704)	23-460 (285)
Tiki Island	23-460 (219)	<3-240 (127)
Yacht Club	9-9 (9)	<2-<2 (<2)
North Redfish	23-23 (23)	<2-<3 (<3)
South Redfish	4-23 (14)	<2-<3 (<3)
Reef 59	7-43 (21)	<3-4 (4)
Reef 63	<3-15 (7)	<3-<3 (<3)
Bottom sediment		
April Fool's Point	4,600-110,000 (33,086)	<2-1,200 (354)
Mowle's Bait Camp	≥24,000-≥ 240,000 (80,265)	110-28,000 (6,445)
Red Bluff Reef	430-430 (430)	<2-1,300 (651)
Eagle's Point	2,000-≥ 240,000 (54,600)	70-46,000 (9,300)
Tiki Island	1,800-110,000 (27,880)	70-21,000 (3,782)
Yacht Club	11,000-11,000 (11,000)	<2-<2 (<2)
North Redfish	Not done	Not done
South Redfish	11,000-11,000 (11,000)	<30-<30 (<30)
Reef 59	430-2,800 (1,387)	<30-70 (47)
Reef 63	430-15,000 (5,643)	70-430 (197)
Oysters		
April Fool's Point	430-46,000 (8,170)	<2-11,000 (1,758)
Mowle's Bait Camp	790-110,000 (22,930)	<30-46,000 (7,817)
Red Bluff Reef	93-93 (93)	40-920 (480)
Eagle's Point	≥2,400-≥24,000 (17,880)	930-≥24,000 (9,866)
Tiki Island	750-11,000 (5,628)	79-≥2,400 (1,133)
Yacht Club	70-70 (70)	<2-<2 (<2)
North Redfish	210-210 (210)	40-40 (40)
South Redfish	40-150 (95)	40-40 (40)
Reef 59	230-1,500 (773)	<3-750 (218)
Reef 63	430-≥2,400 (1,253)	<30-40 (37)

[a] Range (minimum and maximum values).
[b] Arithmetic mean. Means were calculated assuming that < and ≥ counts were equal to the counts reported.

oyster, and sediment samples all closely paralleled the total coliform numbers of these samples.

The means of total coliform:fecal coliform ratios in water from all 10 sites were between 2 and 8, whereas they were between 8 and 5,500 for sediment samples and between 2 and 41 for oyster samples. This indicates that a large proportion of bacteria in the sediment samples was total coliforms rather than fecal coliforms. This is also supported by the data of Carney et al. (4) and Goyal et al. (19).

Salmonellae. Despite repeated attempts, salmonella organisms were not isolated from any sample during the course of this study.

Virus. Of a total of 30 samples each of water and oysters taken from closed sites, 19 and 12 samples, respectively, yielded virus. From open sites, 7 of 14 100-gallon samples of water were positive, whereas 2 pools (100 g each) of oysters yielded virus out of a total of 10 pools examined (Table 3). Virus was isolated from nine study areas on at least one occasion. One site (South Redfish Reef) was sampled four times but did not yield any virus. On several occasions virus was isolated from 100-gallon samples of overlying water but not from oysters, and vice versa. On nine occasions, however, virus was recovered both from oysters and their overlying waters (Table 3). The numbers of viruses in water varied between 4 and 167 PFU/100 gallons of water, whereas they were between 6 and 224 PFU/100 g of oyster meat (Table 4). Virus was isolated from both water and oyster samples when the MPN (per 100 ml) of total coliforms in water and in oysters were 3 and 70, respectively, and when fecal coliforms were as low as <2 and 2, respectively. Of simultaneously positive water and oyster samples, the number of PFU isolated was higher in five oyster samples as compared to water samples.

Only three types of enteroviruses were iso-

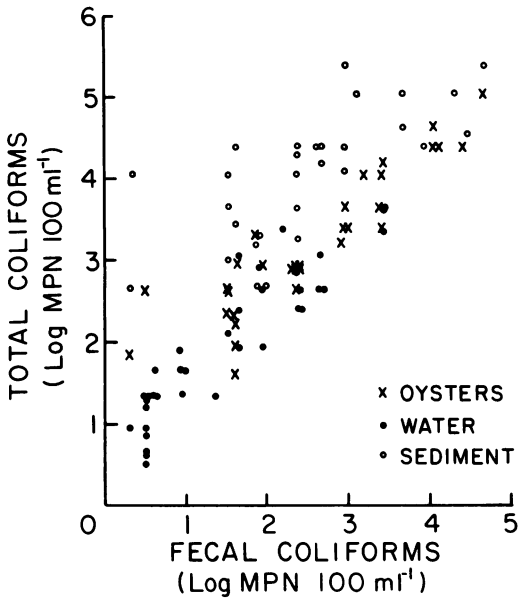

FIG. 1. *Numbers of total and fecal coliforms in water, oyster, and sediment samples.*

lated during this study. These were poliovirus types 1 and 2 and echovirus type 1 (Table 4). All were isolated from both water and oysters. Of the 26 positive water samples, two yielded a mixture of echovirus type 1 and poliovirus type 1. Mixed isolation did not occur in oyster samples. A total of 759 plaques were counted in this study; 258 of these were plucked and 188 were identified by using antisera pools against 42 enteroviruses (28). Poliovirus type 1 was the most common isolate (75.2%) followed by echovirus type 1 (19.6%) and poliovirus type 2 (5.2%). About 20% of the poliovirus isolates were examined by the delayed marker and temperature tests, and all were found to be of vaccine origin.

Statistical analysis. The results of statistical analysis of data are given in Table 5. The following relationships were significant at $P < 0.005$. The MPN of total and fecal coliforms in water and oyster samples were very strongly correlated with each other. The total coliforms in sediments were not correlated with anything except fecal coliforms in sediments. Viruses in oysters were not correlated with any parameter studied, but viruses in water were moderately correlated with total coliforms in water and oysters and with fecal coliforms in oysters. Among physicochemical qualities of water, pH had a slight negative correlation with salinity. A moderate positive correlation was found between rainfall and the bacteriological quality of water. A moderate correlation also occurred between turbidity of water and total and fecal coliform populations of water and oysters.

DISCUSSION

Consumption of raw or improperly cooked shellfish harvested from polluted waters is known to be a vehicle for transmission of hepatitis virus and nonbacterial gastroenteritis. At least 19 outbreaks involving approximately 2,000 cases of shellfish-associated hepatitis have been documented (14). The possibility of transmission of other enteroviruses by shellfish has been recognized, but because of the occult nature of these waterborne viruses no proof of causal relationship between the presence of a pathogen and the outbreak of illness has been established. However, enteroviruses have been isolated from both estuarine waters (9, 20, 34) and shellfish (12, 25, 27, 31, 35, 41), and their potential for disease transmission always exists.

The public health safety of shellfish and shellfish-harvesting waters in the United States is judged presently by bacteriological standards. These standards require that "Most probable numbers of coliforms in water should not exceed 70 per 100 ml and no more than 10% of samples should exceed 230 coliforms per 100 ml in areas approved for harvesting. Shellfish meat in itself is required to contain no more than 230 fecal coliforms per 100 grams" (44). On the basis of these standards, oyster beds have been classified into three categories, i.e., closed, approved, and conditional. However, recent disease outbreaks and field studies (12, 38) have indicated that these may not be adequate indicators of the viral disease hazard.

The present study was undertaken to determine the prevalence of enteroviruses in shellfish and shellfish-harvesting waters from areas both open and closed to shellfish harvesting. It was also intended to determine the occurrence of viruses, indicator bacteria and *Salmonella* in oysters and water samples in relation to the physicochemical characteristics of the water. The results described are the first extensive project in which a substantial amount of quantitative virological data was collected from both open and closed areas and compared with the bacteriological indicators and other environmental factors on a statistical basis.

The populations of total and fecal coliforms were always higher in sediment and oyster samples than in the overlying waters (Table 2). Previous studies conducted by our laboratory along the upper Texas Gulf coast also indicated that marine sediments may serve as significant reservoirs of bacteria and enteric viruses dis-

TABLE 3. *Isolation of viruses as compared to bacterial numbers*

Station	Mean MPN of total coliforms per 100 ml or 100 g of			Mean MPN of fecal coliforms per 100 ml or 100 g of:			Virus isolations in:		
	Water	Sediment	Oysters	Water	Sediment	Oysters	Water (no. positive/no. examined)	Oysters (no. positive/no. examined)	Both positive
Closed to shellfish harvesting									
April Fool's Point	472	33,086	8,170	46	354	1,758	6/9	6/9	4
Mowle's Bait Camp	1,179	91,000	22,930	421	6,445	7,817	3/6	2/6	0
Red Bluff Reef	23	430	93	6	650	480	2/2	0/2	0
Eagle's Point	704	54,600	17,880	285	9,300	8,222	5/6	1/6	1
Tiki Island	219	27,880	5,628	127	3,782	1,133	3/6	2/6	2
Yacht Club	9	11,000	70	0	0	0	0/1	1/1	0
Total							19/30 (63)[a]	12/30 (40)	
Open to shellfish harvesting									
North Redfish	23	ND[b]	210	2	ND	40	2/3	0/1	0
South Redfish	14	11,000	95	3	30	40	0/4	0/2	0
Reef 59	21	1,387	773	4	47	218	3/4	1/4	1
Reef 63	4	1,500	930	3	90	40	2/3	1/3	1
Total							7/14 (50)	2/10 (20)	
All sites	412	36,855	8,729	130	3,308	3,100	26/44 (59)	14/40 (35)	9

[a] Data in parentheses are percents.
[b] ND, Not done.

TABLE 4. *Identification of enterovirus isolates from field samples of water and oysters*[a]

Station	Enterovirus isolates from 100-gallon (~400-liter) samples of water						Enterovirus isolates from 100-gram pools of oyster meat					
	No. positive/no. examined	No. of isolates			Total no. of isolates	Range[b]	No. positive/no. examined	No. of isolates			Total no. of isolates	Range[c]
		Poliovirus type 1	Poliovirus type 2	Echovirus type 1				Poliovirus type 1	Poliovirus type 2	Echovirus type 1		
Closed to shellfish harvesting												
April Fool's Point	6/9	60[d]	0[e]	70	130	27-49	6/9	100	0	2	102	6-205
Mowle's Bait Camp	3/6	41	0	35	76	29-88	2/6	11	0	3	14	16-22
Red Bluff Reef	2/2	32	0	0	32	37-49	0/2	0	0	0	0	0
Eagle's Point	5/6	57	0	2	59	4-30	1/6	57	0	0	57	224
Tiki Island	3/6	36	0	31	67	13-34	2/6	41	0	0	41	21-93
Yacht Club	0/1	0	0	0	0	0	1/1	9	0	0	9	18
Open to shellfish harvesting												
North Redfish	2/3	48	0	0[f]	48	7-167	0/1	0	0	0	0	0
South Redfish	0/4	0	0	0	0	0	0/2	0	0	0	0	0
Reef 59	3/4	11	39	0	50	6-49	1/4	23	0	0	23	59
Reef 63	2/3	45	0	0	45	32-60	1/3	0	0	6	6	17
All sites	26/44	330	39	138	507	—	14/40	241	0	11	252	—

[a] A total of 188 isolates were identified.
[b] PFU per 100-gallon sample.
[c] PFU per 100 g of oyster meat.
[d] Mixed infection. Both echo 1 and polio 1 were isolated by plaquing.
[e] No virus isolated by plaquing. Polio 2 was isolated by the cytopathogenic procedure.
[f] Mixed infection. Polio 1 was isolated by plaquing and echo 1 and polio 1 were isolated by the cytopathogenic procedure.

charged from sewage outfalls (15, 16, 19). Because oyster beds are located on the bottom of the body of water and because the water-sediment interface is not a static system, sediments might act as significant reservoirs of pollution for oysters.

No *Salmonella* was isolated in the present study. This was surprising because in a previous

TABLE 5. *Product-moment correlation coefficients of all measured parameters*

Determination	Rainfall	pH	Salinity	Turbidity	Site[a]	Temperature	Organics	TCW[b]	TCS[b]	TCO[b]	FCW[c]	FCS[c]	FCO[c]	VW[d]	VO[d]
Rainfall	1.0000														
pH	0.1507	1.0000													
Salinity	0.0130	−0.4226[e]	1.0000												
Turbidity	−0.3173	−0.3396	0.0651	1.0000											
Site	0.6213[e]	0.4036	−0.2027	−0.5268[e]	1.0000										
Temp	−0.1446	−0.1564	0.1913	0.0804	−0.3097	1.0000									
Organics	−0.2269	−0.0770	−0.1558	0.7145[e]	−0.3698	−0.1019	1.0000								
TCW	−0.2119	−0.3045	−0.0992	0.4917[e]	−0.2938	−0.1179	0.0725	1.0000							
TCS	−0.1915	−0.0914	0.0168	−0.0444	−0.2831	0.3224	−0.0362	0.2215	1.0000						
TCO	−0.1749	−0.3517	−0.0900	0.4904[e]	−0.2555	−0.1056	0.0325	0.9481[e]	0.1259	1.0000					
FCW	−0.1394	−0.2663	0.0237	0.5129[e]	−0.2128	0.0966	0.0472	0.8724[e]	0.0577	0.9413[e]	1.0000				
FCS	−0.1169	−0.1659	0.0131	−0.0172	−0.1711	0.1200	−0.0397	0.1079	0.5996[e]	0.0746	0.1054	1.0000			
FCO	−0.1605	−0.2144	0.1005	0.4390[e]	−0.2106	0.0609	−0.0000	0.8807[e]	0.0333	0.9423[e]	0.9388[e]	0.0531	1.0000		
VW	0.1113	0.1821	−0.0083	0.1085	0.0549	−0.1993	0.0606	0.5079[e]	0.0509	0.5113[e]	0.2687	−0.1733	0.4616[e]	1.0000	
VO	−0.1803	0.1494	−0.0110	0.0244	−0.1663	−0.1587	0.0001	−0.1047	−0.0180	−0.0996	−0.0859	−0.0426	−0.0685	0.0329	1.0000

[a] Sites were classified into open and closed areas.
[b] TCW, TCS, and TCO, Total coliforms in water, sediment, and oysters, respectively.
[c] FCW, FCS, and FCO, Fecal coliforms in water, sediment, and oysters, respectively.
[d] VW and VO, Viruses in water and oysters, respectively.
[e] Significant at $P < 0.005$.

study we had isolated salmonellae from 17 of 36 sediment samples and from 1 of 36 water samples from shallow coastal waters in areas near sewage outfalls. However, Metcalf et al. (30) could not isolate salmonellae from bay collecting stations, although the number of salmonellae isolated from stations in proximity to the sewage outfall was always greater than enterovirus isolates. They also found that the occurrence of salmonellae decreased proportionately with the distance of the collecting station from the pollution source, whereas enterovirus isolations did not show this trend.

Coliform organisms have been used as indicators of the sanitary quality of water for over 70 years and also are used to classify shellfish-harvesting areas. The isolation of viruses from water and shellfish samples taken from approved areas (Table 3), however, indicates that coliforms are not inviolable indicators of viruses. Viruses were isolated from water and oyster samples on occasions when the MPN of total and fecal coliforms were as low as 3/100 ml of water. Viruses were isolated from 12 water samples and 8 oyster samples when total coliform counts in water samples met the accepted standards. Similarly, 14 samples of water and 7 samples of oysters were positive for virus when the MPN of fecal coliforms in oysters met the current bacteriological standards for shellfish harvesting.

That coliforms are not unequivocal indicators of virological quality of water or shellfish has been demonstrated on previous occasions. In a study conducted along the Texas Gulf coast, we demonstrated the presence of enteroviruses in water in the absence of detectable fecal coliforms (20). Enteric viruses also have been detected in oysters taken from Galveston Bay, an area that met bacteriological standards for shellfish harvesting (12), and from beaches with no detectable fecal coliforms (41). That accepted bacteriological surveillance methods cannot ensure the virological safety of a particular water source also was demonstrated by an epidemic of hepatitis A in the south-central portion of the United States which was caused by the consumption of Gulf coast oysters that met bacteriological standards (26).

Of a total of 759 viral isolates, 188 were identified by using antiserum pools against 42 enteroviruses (28). Only three viral types were encountered, poliovirus type 1 being the most common (75%) (Table 4). This is not surprising because our methods for virus concentration and detection have been developed by using poliovirus as a model and hence are optimal for concentration of this virus. Twenty-six poliovirus isolates were examined by the delayed and temperature marker tests (3, 22), and all were found to have the markers of a vaccine virus, which is not unexpected because oral poliovaccine is used routinely to vaccinate young children in the area.

The isolation of different virus types from the same site over different sampling times may be due to fluctuating patterns of infection in a given community. The number and type of enteroviruses detectable in sewage have been found to vary from month to month and from one community to another. Hence, the change in the type of virus isolated from a given site over a period of several months may reflect the virus type circulating in that community, whether recognized or not, at any given time. Thus, echovirus type 7, which was found to be endemic in Houston on previous occasions (21, 35, 43), was not isolated on a single occasion in the present study.

On several occasions viruses were isolated from simultaneously collected samples of water and oysters, but on other occasions they were isolated only from one or the other (Table 3). Also, when viruses were isolated simultaneously from both water and oyster samples, the type of virus isolated from water sometimes differed from that isolated from the oysters (Table 4). This may be due to the fluctuating nature of the water quality within an estuarine environment, which is greatly influenced by both tidal and fresh water inflow into the estuary. Thus, viruses could be retained by shellfish even after sewage pollution is no longer evident.

Metcalf et al. (35) carried out parallel examinations of oysters and their overlying seawater in Galveston Bay for a period of 3 months for the presence of fecal coliforms and enteroviruses. On seven occasions, water samples of 25 to 105 gallons (ca. 94.6 to 397.4 liters) were processed, but no virus was isolated. In simultaneously collected oyster samples, however, poliovirus was found on two occasions.

Recently, a change from a total to a fecal coliform standard has been proposed because fecal coliforms are considered to be better indicators of fecal pollution. The proposed standards require that "the fecal coliform median MPN of shellfish growing water shall not exceed 14 per 100 ml and not more than 10 percent of the samples shall exceed an MPN of 43 for a 5-tube decimal dilution test or 49 for a 3-tube decimal dilution test" (23). In the present study, total coliform numbers in water, sediment and oyster samples were correlated very strongly to fecal coliform numbers in the same samples. Also, total coliforms in water were correlated with

total and fecal coliforms in oysters (Table 5). These observations suggest the validity of the proposed revised standards, which require the determination of fecal coliforms in water and oysters as opposed to the present standards which require determination of total coliforms in water and fecal coliforms in oysters.

None of the parameters studied was found to correlate with the presence of viruses in oysters (Table 5). A moderate statistical correlation was observed between viruses in water and MPN of total coliforms in water, total coliforms in oysters, and fecal coliforms in oysters. In a previous study, viruses in water were found to be correlated only to total coliforms in sediments (20). That study was performed in shallow coastal canals in the proximity of waste discharges, where sediments were probably contaminated with domestic and other organic-laden wastes, allowing for longer bacterial survival (16). A common fact that emerges from both these studies is that total coliforms might be better indicators of viral pollution of water than fecal coliforms. More extensive studies, however, need to be done to either prove or disprove this point.

The negative correlation between pH and salinity and the positive correlation observed between total and fecal coliforms in water, sediment, and oyster samples are not unexpected. The observed relationship between rainfall and the pollution status of the site may be due to the release of sediment-bound bacteria into the water column of polluted areas because of disturbance of sediments after rainfall. A strong positive correlation between turbidity and organics might be due to the increased output of organics by wastewater, which also results in increased turbidity of the receiving waters.

In view of the great protein shortage in the world, shellfish are and will continue to assume nutritive importance. Clearly, the shellfish industry and the shellfish-consuming public must be protected from the threat of illness resulting from shellfish harvesting and consumption. Results obtained in this study indicate the desirability of re-evaluating the current bacteriological standards. Also, information is needed on the relationship of sediment-associated virus and the safety of recreational water, shellfish, and shellfish-harvesting water.

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