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Distribution of *Vibrio cholerae* in the Apalachicola (Florida) Bay Estuary

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ABSTRACT

Vibrio cholerae non-O1 was found throughout the Apalachicola, FL, estuary. *V. cholerae* O1 was isolated primarily at the City of Apalachicola sewage treatment plant, Scipio Creek and the north shore of St. George Island. Highest concentrations of both serogroups occurred in August and November. Concentrations were lowest in February and increased substantially in May. A cholera toxin-like toxin was not detected in any of the *V. cholerae* cultures by the Y-1 mouse adrenal cell assay or the enzyme-linked immunosorbent assay (ELISA). However, 35% of the *V. cholerae* O1 and 22% of the *V. cholerae* non-O1 cultures selected for testing caused diarrhea in infant rabbits. The proportion of *V. cholerae* O1 and non-O1 isolates pathogenic to infant rabbits increased as water temperature decreased. Fecal coliforms appeared to be more useful than total coliforms as indicators of the numbers of *V. cholerae* in water, but neither of those National Shellfish Sanitation Program indicator groups ensured against the presence of pathogenic *V. cholerae* in shellfish-growing areas.

Several seafood-associated *Vibrio cholerae* cases along the U.S. Gulf Coast have been reported in recent years (*2,3,8,12*). A bacteriological survey of the area indicated the widespread occurrence of *V. cholerae* serogroup non-O1 in shellfish-growing areas (*5*). Oysters harvested from National Shellfish Sanitation Program (NSSP)-approved growing areas in Apalachicola Bay, FL, have been associated with human gastroenteritis caused by both *V. cholerae* O1 (*16*) and *V. cholerae* non-O1 (*19*).

Apalachicola Bay is located in northwest Florida and is about 50 km east to west and 10 km north to south with an average depth of 1 m. The water quality of the Bay is influenced to a large degree by the Apalachicola River, which is 173 km long, drains 50,500 sq. km and has an annual mean flow of 675,000 L/s (*11*). A series of barrier islands separates the Bay from the Gulf of Mexico. Inlets between these islands permit the exchange of saline Gulf water with the lower salinity Bay water, providing an excellent habitat for shellfish.

The Apalachicola Bay harvest of oysters constitutes 90% of Florida's production. However, various domestic, industrial and agricultural pollution sources make oyster harvest from some Bay areas prohibitive. Areas subject to periodic flooding by the Apalachicola River are conditionally approved for shellfish harvesting. The Apalachicola sewage treatment plant may also contribute to the contamination of shellfish-growing waters. Other contamination sources include septic tank leachate, storm sewers, boats, seafood-processing wastes, wildlife and livestock.

The objectives of this study were to determine the distribution, possible sources and pathogenicity of *V. cholerae* in the Apalachicola estuary. The data were analyzed to determine whether the levels of fecal and total coliforms were indicative of the presence of *V. cholerae*. We have previously noted the isolation of pathogenic *V. cholerae* O1 Ogawa in NSSP-approved waters in Apalachicola Bay (*13*) and the isolation of *Vibrio parahaemolyticus* from raccoons in Florida (*4*).

MATERIALS AND METHODS

Sample collection

Water samples were collected quarterly from November 1980 to August 1981 from 22 sampling stations (Fig. 1). Stations 7 (Apalachicola River upstream from Jackson River), 8 (Jackson River, Intracoastal Waterway) and 9 (Apalachicola River downstream from Jackson River at fishing camp) were chosen to assess potential *V. cholerae* sources in the Apalachicola and Jackson Rivers. Station 11 was located at the City of Apalachicola sewage treatment plant, which serves 750 dwellings and discharges into Scipio Creek. Stations 3, 4, 5 and 6 were located in the Creek to track sewage treatment plant effluent. Special stations were chosen to evaluate nearby pollution sources at stations 12 (Howard's Creek, cattle ranch), 10 (Ten-Foot Hole, storm sewers and boats), 14 (Cash Creek, wildlife), 13 (East Bay, fishing camp), 19 (East Point, seafood processing and septic tanks), 17 (St. George Island, marina), 2 (St. Vincent Island, National Wildlife Refuge,

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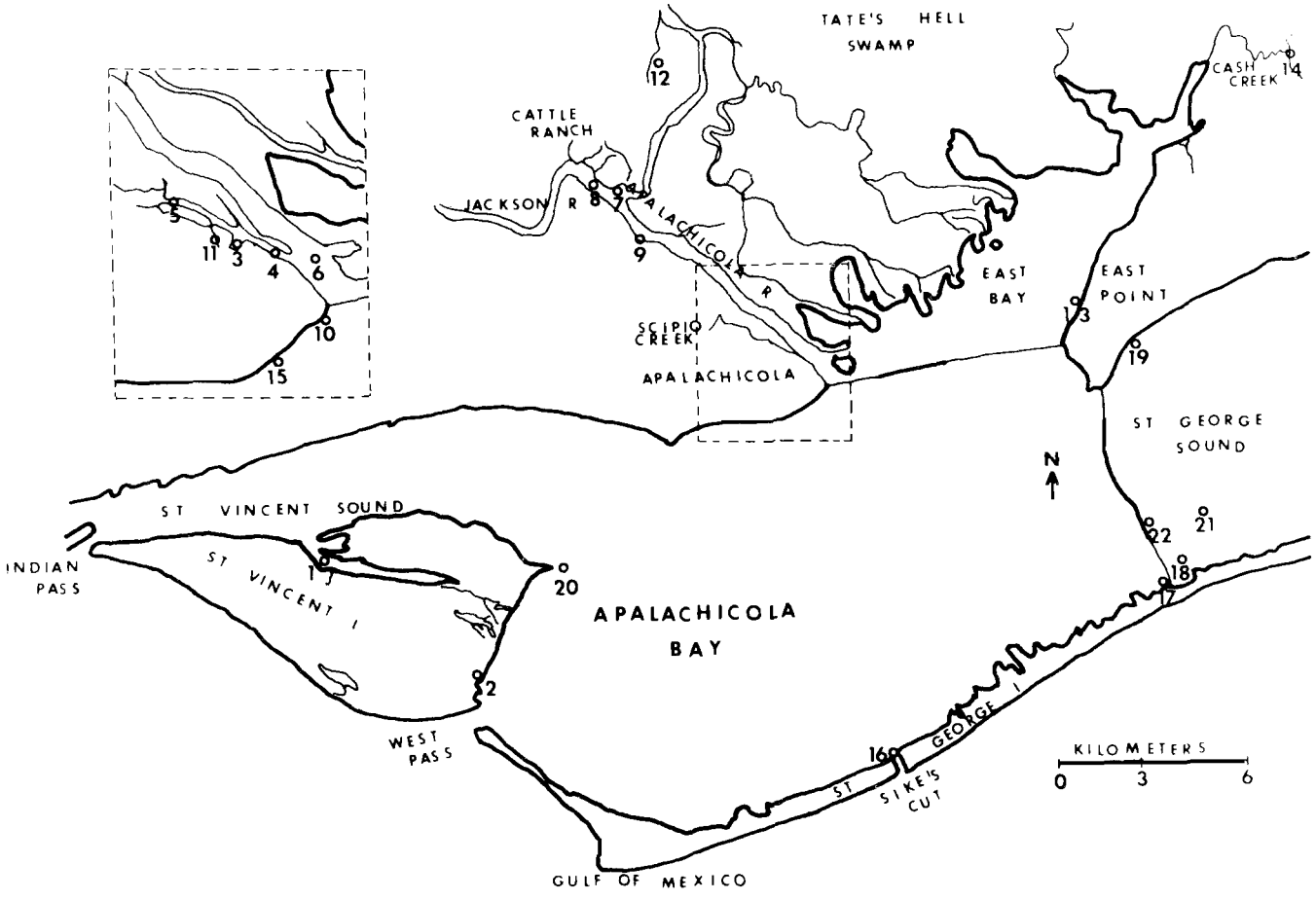

Figure 1. *Sampling stations.*

wildlife) and 15 (Two-mile channel, seafood processing and septic tanks). Stations 1 (Big Bayou, St. Vincent Island), 20 (Dry Bar), 18, 21 and 22 (East Hole) were at commercially important oyster reefs. Station 16 (Sike's Cut) was nearest to the Gulf and was remote from any known pollution sources.

Most stations were sampled twice on successive days each quarter. Water samples were collected in 4-L Nalgene containers, using American Public Health Association (APHA) procedures (*1*). The samples were chilled with ice and analyzed within 6 h of collection. Sample station water temperature was measured to the nearest degree C and bottom and surface salinities in parts per thousand (ppt) were measured with a Beckman induction salinometer RS 5-3 or an American Optical Corporation hand refractometer.

Bacteriological analyses

The most probable number (MPN) analysis of water samples of *V. cholerae,* which was similar to that recommended in the *Bacteriological Analytical Manual (7),* is described by DePaola et al. (*5*). Total and fecal coliform values were also determined by a 5-tube MPN technique (*1*). Total coliform MPNs were determined with lauryl sulfate tryptose (LST) broth (Difco) incubated at 35°C for 24 and 48 h and confirmed in brilliant green broth (Difco) at 35°C for 24 h. Fecal coliform MPNs were based on LST and confirmed with EC broth (Difco) incubated 24 h at 44.5°C.

Toxicity and pathogenicity

All *V. cholerae* serogroup O1 isolates and 10% of the *V. cholerae* non-O1 isolates selected at random were tested for toxigenicity and pathogenicity. Culture filtrates were screened for a cholera toxin (CT)-like activity by an enzyme-linked immunosorbent assay (ELISA) method (*20*) and by the Y-1 mouse adrenal cell technique (*18*). Pathogenicity determinations were based on the ability of an organism to induce diarrhea in infant rabbits (8 to 10 d) when cell concentrations of 10^9 were injected into the ileum (*6*).

Typing of V. cholerae *O1*

Cultures exhibiting biochemical ultilization patterns of *V. cholerae* were tested for agglutination in O group 1 antiserum. Those showing agglutination were further tested in Ogawa and Inaba antisera, which were kindly provided by Dr. Harry Smith of the *Vibrio* Reference laboratory at Thomas Jefferson Medical College. Phage typing was done by Dr. John Lee at the Maidstone Public Health Laboratory, using the phages of Mukerjee (*14*).

Analysis of data

Data were analyzed by the Division of Mathematics, Food and Drug Adminstration (FDA), by the General Linear Models Procedure (SAS Institute, Cary, NC).

RESULTS

V. cholerae organisms were detected in 57% of 150 water samples examined. Of 753 cultures identified as *V. cholerae* by biochemical utilization patterns, 733 were classified as *V. cholerae* non-O1, based on failure to agglutinate in O group 1 antiserum; 20 were classified as *V. cholerae* O1. More specific serological testing of these 20 *V. cholerae* O1 cultures indicated that 8 were *V. cholerae* O1 Ogawa and 12 were *V. cholerae* O1 Inaba. A CT-like toxin was not detected in any of the 20 *V. cholerae* O1 cultures or in 77 randomly selected

non-O1 cultures by either the Y-1 mouse adrenal cell assay or the ELISA. However, 35% of the *V. cholerae* O1 and 22% of the 77 non-O1 cultures caused diarrhea in infant rabbits. The phage types of the O1 cultures were variable and differed from those of all recent clinical O1 isolates (P. A. Blake, Centers for Disease Control, personal communication).

Seasonality of V. cholerae

Table 1 illustrates the seasonality of *V. cholerae*. Highest *V. cholerae* concentrations occurred in August; the lowest were found in February, with intermediate levels in November and May. Similar mean fecal and total coliform MPNs and salinities were recorded at each sampling time. The proportion of *V. cholerae* O1 and non-O1 isolates pathogenic to infant rabbits increased as water temperature decreased.

Geographical distribution of V. cholerae

Mean physical and bacteriological data for the various sample station grouping are listed in Table 2. *V. cholerae* non-O1 was found throughout the Apalachicola estuary, but *V. cholerae* O1 was primarily isolated at Scipio Creek and the north shore of St. George Island. Moderately high fecal coliform concentrations were observed in the fresh water of the Apalachicola River, where *V. cholerae* concentrations were low. High *V. cholerae* concentrations were found in Scipio Creek at stations 3, 4 and 5 and were similar to those observed at the sewage treatment plant (station 11), whereas fecal and total coliform concentrations were much higher at the sewage treatment plant.

High fecal and total coliform and *V. cholerae* concentrations were observed at stations 2, 10, 13 and 14. Fecal and total coliform and *V. cholerae* concentrations were low for stations 18, 20, 21 and 22, which are commercially important oyster reefs.

NSSP fecal and total coliform standards

In Table 3, fecal coliform MPNs are grouped according to NSSP guidelines for classifying shellfish-growing areas. Under the guidelines, harvesting of shellfish is prohibited if the median fecal coliform MPN of waters is >14/100 ml or if 10% of the samples are >43/100 ml (*9*). Water samples exceeding these standards contained significantly higher ($P<0.05$) *V. cholerae* concentrations and a higher ratio of pathogenic non-O1 isolates than samples meeting the standards. A higher ratio of pathogenic *V. cholerae* O1 isolates was found in samples meeting the fecal coliform standard; however, this difference was not significant.

In Table 4, groupings were based on NSSP total coliform guidelines for classifying shellfish-growing areas.

TABLE 1. *Seasonal distribution of numbers and pathogenicity of* V. cholerae *in Apalachicola water samples.*

Month	No. of samples	Temp. (°C)	*V. cholerae* MPN/100 ml (log_{10})	% Positive for *V. cholerae*	Pathogenicity ratio[a] Non-O1 isolates	Pathogenicity ratio[a] O1 isolates
November	35	18[d]	1.5[c]	77[f]	10/28[g]	2/6[g]
February	38	10[e]	-0.6[d]	23[g]	2/3[f]	3/3[f]
May	38	24[c]	1.0[c]	66[f]	2/16[h]	1/4[g]
August	39	30[b]	2.2[b]	64[f]	3/30[h]	1/7[g]

[a]Pathogenicity based on ability of culture to cause diarrhea in infant rabbits and expressed as number of cultures causing diarrhea/total number tested.

[b,c,d,e]Mean values in same column followed by common letter are not significantly different ($P<0.05$) by Duncan's mean separation technique.

[f,g,h]Mean values in same column followed by common letter are not significantly different ($P<0.05$) by normal deviate Z.

TABLE 2. *Geographical distribution of salinity,* V. cholerae *and indicator organisms.*

Area	Stations	No. of Samples	Salinity[a] (ppt)	% Positive for *V. cholerae*	No. of O1 isolates	MPN/100 ml (log_{10}) *V. cholerae*	MPN/100 ml (log_{10}) Fecal coliform	MPN/100 ml (log_{10}) Total coliform
Apalachicola River	7,8,9,12	25	0	44	0	0.1	1.4	2.5
Apalachicola sewage treatment plant	11	8	0	50	4	2.3	5.9	6.3
Apalachicola City	3,4,5,6,10,15	48	2	79	8	2.1	2.2	3.4
Cash Creek	14	8	7	50	0	0.8	2.1	3.1
East Bay	13	8	12	87	0	2.7	2.6	2.9
East Point	19	8	25	37	0	0.4	1.6	2.4
St. George Island	17,18,21,22	22	26	27	5	-0.2	0.5	0.9
Sike's Cut	16	7	30	29	2	-0.2	0.3	0.4
Apalachicola Bay	20	4	19	50	0	0.1	0.3	1.2
St. Vincent Island	1,2	12	18	75	1	1.5	1.5	1.6

[a]Expressed as parts per thousand (ppt).

TABLE 3. *Distribution of* V. cholerae *numbers, frequency and pathogenicity in NSSP-approved and prohibited shellfish-growing waters by fecal coliform standard.*

Fecal coliform range (MPN/100 ml)	*V. cholerae* MPN/100 ml (log_{10})	% Positive for *V. cholerae*	Pathogenicity ratio[a]	
			Non-O1 isolates	O1 isolates
≤14	0.1[c]	38[e]	2/16[d]	4/7[d]
>14	1.5[b]	68[d]	15/61[d]	3/13[d]
≤43	0.4[c]	46[e]	3/26[e]	4/8[d]
>43	1.6[b]	68[d]	14/51[d]	3/12[d]

[a]Pathogenicity based on ability of culture to cause diarrhea in infant rabbits and expressed as number of cultures causing diarrhea/total number tested.
[b,c]Mean values in same column followed by common letter are not significantly different ($P<0.05$) by Duncan's mean separation technique.
[d,e]Mean values in same column followed by common letter are not significantly different ($P<0.05$) by normal deviate Z.

TABLE 4. *Distribution of* V. cholerae *numbers, frequency and pathogenicity in NSSP-approved and prohibited shellfish-growing waters by total coliform standard.*

Total coliform range (MPN/100 ml)	*V. cholerae* MPN/100 ml (log_{10})	% Positive for *V. cholerae*	Pathogenicity ratio[a]	
			Non-O1 isolates	O1 isolates
≤70	0.4[c]	38[e]	4/19[d]	4/8[d]
>70	1.3[b]	68[d]	13/58[d]	3/12[d]
≤230	0.5[c]	46[e]	4/30[d]	4/8[d]
>230	1.5[b]	68[d]	13/47[d]	3/12[d]

[a]Pathogenicity based on ability of culture to cause diarrhea in infant rabbits and expressed as number of cultures causing diarrhea/total number tested.
[b,c]Mean values in same column followed by common letter are not significantly different ($P<0.05$) by Duncan's mean separation technique.
[d,e]Mean values in same column followed by common letter are not significantly different ($P<0.05$) by normal deviate Z.

Under the guidelines, shellfish harvesting is prohibited if the median total coliform MPN of waters is >70/100 ml or if 10% of the samples are >230/100 ml (*17*). The same trend appears as with fecal coliform groupings, except that differences in frequency of *V. cholerae* are less distinct with total coliform groupings.

DISCUSSION

The incidence and seasonal variations of *V. cholerae* non-O1 in the Apalachicola estuary were similar to those observed in other estuaries along the U.S. Gulf Coast (*5*). Although *V. cholerae* O1 was isolated more frequently in Apalachicola than at other Gulf Coast locations, the public health significance of the *V. cholerae* O1 isolates is debatable. A CT-like toxin was not detected; however, 35% of the isolates produced diarrhea in infant rabbits.

The highest concentrations of *V. cholerae* were recovered in August and the next highest in late November, whereas the greatest proportion of *V. cholerae* O1 and non-O1 pathogenic strains was found in February and November. Nearly all oyster-related infections of *V. cholerae* reported in Florida occurred during November and December (*16,19*), which are also months of peak oyster harvest. The associations imply that late fall and early winter months are the period of greatest public health risk.

The fact that the only substantial numbers of *V. cholerae* in the Apalachicola River were found downstream from Scipio Creek suggests little influence from distant freshwater runoff. The occurrence of *V. cholerae* O1 and non-O1 serogroups at the sewage treatment plant also suggests the presence of human carriers in Apalachicola. Apparently, there were other sources of *V. cholerae* in Scipio Creek, since the highest concentrations were detected upstream from the sewage treatment plant in the vicinity of an extensive marsh not inhabited by humans. Recent hydrographic studies (*15*) have demonstrated that Scipio Creek is unlikely to influence the water quality of any commercially important oyster reefs of Apalachicola Bay. High concentrations of *V. cholerae* at some special sampling sites indicate the need for further investigations into the roles of local storm drainage, septic tank leachate, seafood processing by-products and wildlife in the life cycle of *V. cholerae*.

V. cholerae and fecal coliform concentrations were generally low at commercially important oyster reefs. Pathogenic *V. cholerae* O1 and non-O1 organisms were isolated at station 18. Oysters from this area have been implicated in recent *V. cholerae* O1 and non-O1 cases (*16,19*). A sanitary survey of St. George Island should be conducted to evaluate possible sources of *V. cholerae*.

Although fecal coliforms appear to be more useful than total coliforms as indicators of *V. cholerae* numbers in water, neither of the NSSP indicator groups ensured against the presence of pathogenic *V. cholerae* in shellfish-growing areas. It would be difficult to provide

absolute protection of the public health against *V. cholerae,* since *V. cholerae* is a naturally occurring organism (*10*). The use of additional indicators in conjunction with fecal coliforms may improve the classification criteria, especially at high risk times. More information on the ecology of *V. cholerae* and better recovery methods are needed to formulate a rational regulatory approach.

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