APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1990, p. 2299–2302
0099-2240/90/082299-04$02.00/0

Vol. 56, No. 8

Incidence of *Vibrio parahaemolyticus* in U.S. Coastal Waters and Oysters

ANGELO DEPAOLA,[1]* LINDA H. HOPKINS,[2] JAMES T. PEELER,[3] BARRY WENTZ,[3] AND ROLAND M. MCPHEARSON[1]

Fishery Research Branch, U.S. Food and Drug Administration, Dauphin Island, Alabama 36528[1]; Department of Biology, Jacksonville State University, Jacksonville, Alabama 36265[2]; and Division of Microbiology, U.S. Food and Drug Administration, Washington, D.C. 20204[3]

Received 4 April 1990/Accepted 22 May 1990

Oyster and seawater samples were collected seasonally from May 1984 through April 1985 from shellfish-growing areas in Washington, California, Texas, Louisiana, Alabama, Florida, South Carolina, Virginia, and Rhode Island which had been designated as approved or prohibited by the National Shellfish Sanitation Program. Fecal coliforms counts, aerobic plate counts, and *Vibrio parahaemolyticus* densities were determined for the samples. Mean *V. parahaemolyticus* density was more than 100 times greater in oysters than in water, whereas density of fecal coliforms was approximately 10 times higher in oysters. Seasonal and geographical distributions of *V. parahaemolyticus* were related to water temperature, with highest densities in samples collected in the spring and the summer along the Gulf coast. The synthetic DNA probe for thermostable direct hemolysin hybridized with 2 of 50 isolates, 1 of which was positive by the Kanagawa test.

Vibrio parahaemolyticus is an enteric pathogen transmitted to humans primarily through consumption of raw or mishandled seafood (2, 23). Like other members of the genus *Vibrio*, it is a gram-negative, halophilic bacterium that occurs naturally in estuarine environments (15). Its distribution is worldwide, but reported densities of *V. parahaemolyticus* in the environment and in seafood vary greatly according to season, location, sample type, fecal pollution, and analytical methodology (3, 5, 8, 16, 27). The public health significance of *V. parahaemolyticus* contamination in seafoods is debatable, since few environmental or seafood isolates produce the thermostable-direct hemolysin (TDH) or Kanagawa hemolysin associated with most (96.5%) pathogenic strains found among clinical isolates (12, 20, 21). However, a TDH-related hemolysin has been isolated from Kanagawa phenomenon-negative *V. parahaemolyticus* strains associated with gastroenteritis in Southeast Asia (13).

In an evaluation (5) of four methods for the enumeration of *V. parahaemolyticus* in seawater and oysters, we found that the hydrophobic grid membrane filter method described by Entis and Boleszczuk (9) gave the highest density estimates. This method had been used to evaluate the effects of thermal stress on artificially contaminated seafoods but had not been used in environmental surveys. Past *V. parahaemolyticus* surveys were conducted regionally but did not compare distribution of the organism among the Pacific, Gulf, and Atlantic coasts. The present study reports the incidence of *V. parahaemolyticus* in U.S. oysters and seawater as determined by the hydrophobic grid membrane filter method.

The National Shellfish Sanitation Program classification of shellfish-growing areas is based on fecal coliform densities in the overlying waters (14); however, a statistical evaluation is needed to correlate *V. parahaemolyticus* densities in shellfish with either fecal coliform or *V. parahaemolyticus* counts for the overlying waters. In shellfish, *V. parahaemolyticus* counts are much higher than those for overlying waters (3, 5, 7, 8). Thus, the objectives of this study were to compare Pacific, Gulf, and Atlantic coast waters and oysters for densities of *V. parahaemolyticus*; to correlate *V. parahaemolyticus* counts in oysters and water with water salinity, temperature, and bacteriological parameters; and to determine the virulence markers of selected *V. parahaemolyticus* isolates.

MATERIALS AND METHODS

Sample collection. Water and oysters were collected seasonally from May 1984 through April 1985 from 18 locations (two sites in each of nine states), including National Shellfish Sanitation Program-approved and -prohibited areas, in Rhode Island, Virginia, South Carolina, Florida, Alabama, Louisiana, Texas, and Washington. Both sites in California were National Shellfish Sanitation Program-approved areas. Oysters were *Crassostrea virginica* from the Atlantic and Gulf coasts and *Crassostrea gigas* from the Pacific coast.

Water was collected by the method of the American Public Health Association (1) in sterile 1-liter wide-mouth containers (Nalgene Labware Div., Nalge/Sybron Corp., Rochester, N.Y.). Oysters were hand-picked or collected with oyster tongs or dredges and held in insulated containers with ice packs (2 to 5°C); examination was initiated within 48 h (usually within 24 h) after collection. Surface water temperature and salinity were determined at each site by using a mercury thermometer and a hand refractometer (American Optical Corp., Buffalo, N.Y.), respectively.

Bacterial analyses. Oysters were scrubbed, shucked, and blended for 60 s (1). Fecal coliform most probable numbers and aerobic plate counts were determined according to American Public Health Association procedures (1). Water was examined for fecal coliform density but not for aerobic plate count. *V. parahaemolyticus* densities were estimated by the hydrophobic grid membrane filter method (9) with the ISO-GRID membrane filter system (0.45-μm pore size; QA

* Corresponding author.

TABLE 1. Comparison of oyster and overlying waters for *V. parahaemolyticus* and fecal coliforms

Organism	Mean density in:		Correlation[a]
	Oyster (cells/100 g)	Water (cells/100 ml)	
V. parahaemolyticus	5,200	17	0.39
Fecal coliforms	100	13	0.37

[a] Bacterial densities of oyster and corresponding water samples significantly correlated at the $\alpha = 0.05$ level. For each value, $n = 65$.

Laboratories Ltd., Toronto, Ontario, Canada). The following portions were analyzed: 0.1, 0.01, and 0.001 g of oyster homogenate and 100, 10, and 1 ml of seawater. The hydrophobic grid membrane filter method was modified to include an in situ test for acid production from galactose (5). Suspect colonies were considered *V. parahaemolyticus* for enumeration purposes if they met the following criteria: positive growth at 42°C, positive for the oxidase test, acid production from galactose, no growth on gelatin medium without NaCl, negative for *o*-nitrophenyl-β-D-galactopyranosidase, and no acid from sucrose. Representative isolates (50 randomly chosen suspect isolates) were tested by the API 20E system (Analytab Products, Plainview, N.Y.) for confirmation of *V. parahaemolyticus* (22).

Assays for virulence markers. Confirmed *V. parahaemolyticus* isolates were tested phenotypically and genotypically for TDH. A synthetic DNA probe (21) was used to identify the TDH gene, and the Kanagawa test (20) was used to assay for TDH production.

Statistical analysis. Geometric means were determined for bacteriological and physical parameters for oysters and water collected from various coasts during various seasons. Specimens with no observable colonies were assigned the minimum observable density on the basis of the volume examined. The Kruskal-Wallis test, a nonparametric rank test, was used to determine and locate significant differences ($\alpha = 0.05$) among coasts and seasons (11). Pearson's correlation coefficients were determined for bacteriological and physical data from oysters and water (24).

RESULTS

Mean *V. parahaemolyticus* and fecal coliform densities in water and oysters were compared (Table 1). Mean fecal coliform and *V. parahaemolyticus* densities in water were similar but not correlated. *V. parahaemolyticus* density was more than 100 times greater in oysters than in water, whereas fecal coliform density was approximately 10 times higher in oysters. Both *V. parahaemolyticus* and fecal coliform levels in oysters were significantly correlated ($\alpha = 0.05$) to those in the overlying waters, but the correlations were not high (0.39 and 0.37, respectively).

Correlation coefficients for *V. parahaemolyticus* densities and physical or bacteriological variables are shown in Table 2. Only water temperature was significantly correlated ($\alpha = 0.05$) with *V. parahaemolyticus* densities in either water or oysters. The seasonal and geographical distributions of *V. parahaemolyticus* shown in Tables 3 and 4, respectively, relate *V. parahaemolyticus* densities to higher temperatures in the spring and summer along the Gulf coast.

The synthetic DNA probe for TDH hybridized with 2 of 50 isolates, and 1 of these was positive in the Kanagawa test.

TABLE 2. Correlation of *V. parahaemolyticus* density in oyster and water samples with physical and bacteriological parameters

Sample	Correlation of density with:			
	Salinity	Temp[a]	Fecal coliform	APC[b]
Oyster	−0.12 (65)[c]	0.52 (61)	0.25 (65)	0.07 (51)
Water	−0.09 (65)	0.50 (61)	0.08 (65)	ND

[a] Significantly correlated with *V. parahaemolyticus* densities at $\alpha = 0.05$.
[b] APC, Aerobic plate count per gram of oyster; ND, not determined.
[c] Numbers in parentheses are numbers of samples.

TABLE 3. Comparison of *V. parahaemolyticus* density by season

Season	No. of samples	Mean *V. parahaemolyticus*		Temp (°C)
		Oyster (cells/100 g)	Water (cells/100 ml)	
Spring	14	16,000[a]	68[a]	25[a]
Summer	20	6,000[a,b]	21[a,b]	24[a]
Fall	17	4,900[a,b]	13[a,b]	16[b]
Winter	14	1,500[b]	4[b]	10[b]

[a,b] Values followed by a different letter within a column are significantly different at $\alpha = 0.05$. The geometric means are shown in the table; however, the nonparametric test of significance (10) was performed on the ranks.

DISCUSSION

Although both fecal coliform and *V. parahaemolyticus* levels in oysters were correlated to corresponding values in the overlying waters, the ratio of *V. parahaemolyticus* in oyster to *V. parahaemolyticus* in water (about 300:1) was much greater than that observed for fecal coliforms in oyster and in water (about 10:1). Initially, this was considered an artifact of different sensitivities for these organisms in oysters and water. In the statistical analyses, samples without observable *V. parahaemolyticus* were assigned the lower limits of detection on the basis of the largest portion examined (i.e., the limits of detection for *V. parahaemolyticus* were 1,000/100 g of oyster and 1/100 ml of water; the limits for fecal coliforms were 18/100 g of oyster and 1.8/100 ml of water). The levels of each factor (e.g., season) were compared by using ranks (11). However, the estimates of geometric mean were affected by the arbitrary choice of lower limits. Thus, a pair of negative samples would give a 1,000-fold-higher *V. parahaemolyticus* density and a 10-fold-higher fecal coliform density in oysters than in water. A second analysis included only sample pairs in which both oysters and water were positive for *V. parahaemolyticus*. Of the total 65 sample pairs, 33 met this criterion. The mean *V.*

TABLE 4. Comparison of *V. parahaemolyticus* density by coast

Coast	No. of samples	Mean *V. parahaemolyticus* density in:		Temp (°C)
		Oyster (cells/100 g)	Water (cells/100 ml)	
Atlantic	20	3,000[a,b]	19[a]	17[b]
Gulf	31	11,000[a]	44[a]	22[a]
Pacific	14	2,100[b]	2[b]	15[b]

[a,b] Values followed by a different letter within a column are significantly different at $\alpha = 0.05$. The geometric means are shown in the table; however, the nonparametric test of significance (10) was performed on the ranks.

parahaemolyticus densities were 18,000/100 g of oyster and 77/100 ml of water, yielding a 233:1 ratio of *V. parahaemolyticus* in oyster to *V. parahaemolyticus* in water.

Possible explanations for the relatively high *V. parahaemolyticus* densities in oysters include enhanced bioconcentration and survival or growth in the oyster. Previous work (15) demonstrated the association of *V. parahaemolyticus* with zooplankton, which may enhance bioconcentration of *V. parahaemolyticus* by the oyster. Growth of *V. parahaemolyticus* in oysters after harvest was unlikely, since the organism survives but does not multiply below 10°C (4). *V. parahaemolyticus* appears to depurate more slowly in the hard-shell clam, *Mercenaria mercenaria* (10), and may be similar to *V. vulnificus*, which appears to be a natural inhabitant of oyster tissue (M. L. Tamplin and A. L. Martin, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, Q85, p. 344). More definitive studies are needed to explain the high *V. parahaemolyticus* densities in oysters.

The positive correlation between *V. parahaemolyticus* density and water temperature and the lack of correlation with salinity, aerobic plate count, and fecal coliform density probably result from the ecology of this organism, as described by Kaneko and Colwell (15). Its distribution appears to be less salinity dependent than that of *V. cholerae* (6, 17) or *V. vulnificus* (18). The correlation between *V. parahaemolyticus* density and temperature in this study parallels that observed in previous investigations (16, 19). Temperature seems to be the major factor in both seasonal and geographical distribution of *V. parahaemolyticus* in shellfish-growing areas.

The incidence of Kanagawa-positive *V. parahaemolyticus* was low and could not be related to experimental variables. Previous surveys of food and environmental samples also have shown few Kanagawa-positive *V. parahaemolyticus* (20, 25). An additional isolate hybridized with the synthetic DNA probe but was not hemolytic in the Kanagawa assay. This isolate may have a TDH-related hemolysin, which has low hemolytic activity on the human and rabbit erythrocytes used in the Kanagawa assay but low homology with the TDH gene (12). Additional testing (e.g., rabbit ileal loop) is necessary to determine the pathogenicity (26) of isolates that are homologous with the TDH gene but lack hemolytic activity.

ACKNOWLEDGMENTS

We thank the following individuals and organizations for their assistance in collecting oysters and seawater: Robert F. Stott and Jerold M. Leitch, Food and Drug Administration (FDA), Seattle, Wash.; Douglas W. Price, California Department of Health Services, Santa Rosa; Norman Rice, Texas Department of Health, Lamarque; Emile Moline and Daniel Mathews, Louisiana Health and Human Resources Administration, New Orleans; Miles L. Motes, FDA, Dauphin Island, Ala.; William Lunsford, Florida Department of Natural Resources, Apalachicola; Roger O. Olmsted, FDA, Atlanta, Ga.; Sis Nunnally, South Carolina Department of Health, Charleston Heights; David Perry, Virginia Department of Health, Whitestone; Robert H. Brands, FDA, Baltimore, Md.; and Jack L. Gaines, FDA, Davisville, R.I. We also appreciate the laboratory support of Mable L. Carter, Fishery Research Branch, FDA, Dauphin Island, Ala.

LITERATURE CITED

1. **American Public Health Association.** 1970. Recommended procedures for the examination of seawater and shellfish, 4th ed. American Public Health Association, Washington, D.C.
2. **Barker, W. H.** 1974. *Vibrio parahaemolyticus* outbreaks in the United States, p. 47–52. *In* T. Fujino, G. Sakaguchi, R. Sakazaki, and Y. Takeda (ed.), International symposium on *Vibrio parahaemolyticus*. Saikon Publishing Co., Ltd., Tokyo.
3. **Bartley, C. H., and L. W. Slanetz.** 1971. Occurrence of *Vibrio parahaemolyticus* in estuarine waters and oysters of New Hampshire. Appl. Microbiol. **21**:965–966.
4. **Cook, D. W., and A. D. Ruple.** 1989. Indicator bacteria and *Vibrionaceae* multiplication in post-harvest shellstock oysters. J. Food Prot. **52**:343–349.
5. **DePaola, A., L. H. Hopkins, and R. M. McPhearson.** 1988. Evaluation of four methods for enumeration of *Vibrio parahaemolyticus*. Appl. Environ. Microbiol. **54**:617–618.
6. **DePaola, A., M. W. Presnell, M. L. Motes, Jr., R. M. McPhearson, R. M. Twedt, R. E. Becker, and S. Zywno.** 1983. Non-O1 *Vibrio cholerae* in shellfish, sediment and waters of the U.S. Gulf coast. J. Food Prot. **46**:802–806.
7. **Earle, P. M., and F. D. Crisley.** 1975. Isolation of *Vibrio parahaemolyticus* from Cape Cod soft-shell clams (*Mya arenaria*). Appl. Microbiol. **29**:635–640.
8. **El-Sahn, M. A., A. A. El-Banna, and A. M. El-Tabey Shehata.** 1982. Occurrence of *Vibrio parahaemolyticus* in selected marine invertebrates, sediment, and seawater around Alexandria, Egypt. Can. J. Microbiol. **28**:1261–1264.
9. **Entis, P., and P. Boleszczuk.** 1983. Overnight enumeration of *Vibrio parahaemolyticus* in seafood by hydrophobic grid membrane filtration. J. Food Prot. **46**:783–786.
10. **Greenberg, E. P., M. Dubois, and B. Palhof.** 1982. The survival of marine vibrios in *Mercenaria mercenaria*, the hard shell clam. J. Food Saf. **4**:113–123.
11. **Hettmansperger, T. P.** 1984. Statistical inference based on ranks. John Wiley & Sons, Inc., New York.
12. **Honda, T., Y. Ni, and T. Miwatani.** 1988. Purification and characterization of a hemolysin produced by a clinical isolate of Kanagawa phenomenon-negative *Vibrio parahaemolyticus* and related to the thermostable direct hemolysin. Infect. Immun. **56**:961–965.
13. **Honda, T., Y. Ni, and T. Miwatani.** 1989. Purification of a TDH-related hemolysin produced by a Kanagawa phenomenon-negative clinical isolate of *Vibrio parahaemolyticus* O6:K46. FEMS Microbiol. Lett. **57**:241–246.
14. **Hunt, D. A., and J. Springer.** 1974. Preliminary report on a comparison of total coliforms and fecal coliform value in shellfish growing area waters and a proposal for a fecal coliform growing area standard, p. 97–104. *In* Proceedings of the 8th National Shellfish Sanitation Workshop. U.S. Department of Health, Education, and Welfare, Washington, D.C.
15. **Kaneko, T., and R. R. Colwell.** 1973. Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. J. Bacteriol. **113**:24–32.
16. **Kaneko, T., and R. R. Colwell.** 1975. Incidence of *Vibrio parahaemolyticus* in Chesapeake Bay. Appl. Microbiol. **30**: 251–257.
17. **Kaper, J. H., H. Lockman, R. R. Colwell, and S. W. Joseph.** 1979. Ecology, serology, and enterotoxin production of *Vibrio cholerae* in Chesapeake Bay. Appl. Environ. Microbiol. **37**: 91–102.
18. **Kelly, M. T.** 1982. Effect of temperature and salinity on *Vibrio* (*Beneckea*) *vulnificus* occurrence in a Gulf coast environment. Appl. Environ. Microbiol. **44**:820–824.
19. **Kelly, M. T., and E. M. Dan Stroh.** 1988. Temporal relationship of *Vibrio parahaemolyticus* in patients and the environment. J. Clin. Microbiol. **26**:1754–1756.
20. **Miyamoto, Y., T. Kato, Y. Obara, S. Akiyama, K. Takizawa, and S. Yamai.** 1969. In vitro hemolytic characteristic of *Vibrio parahaemolyticus*: its close correlation with human pathogenicity. J. Bacteriol. **100**:1147–1149.
21. **Nishibuchi, M., W. E. Hill, G. Zon, W. L. Payne, and J. B. Kaper.** 1986. Synthetic oligodeoxyribonucleotide probes to detect Kanagawa phenomenon-positive *Vibrio parahaemolyticus*. J. Clin. Microbiol. **23**:1091–1095.
22. **Overman, T. L., J. F. Kessler, and J. P. Seabolt.** 1985. Comparison of API 20E, API rapid E, and API rapid NFT for identification of members of the family *Vibrionaceae*. J. Clin. Microbiol. **22**:778–781.
23. **Ray, B., S. M. Hawkins, and C. R. Hackney.** 1978. Method for

the detection of injured *Vibrio parahaemolyticus* in seafoods. Appl. Environ. Microbiol. **35**:1121–1127.
24. **Snedecor, G. W., and W. G. Cochran.** 1967. Statistical methods, 6th ed. Iowa State College Press, Ames.
25. **Thompson, C. A., Jr., C. Vanderzant, and S. M. Ray.** 1976. Serological and hemolytic characteristics of *Vibrio parahaemolyticus* from marine sources. J. Food Sci. **41**:204–205.
26. **Twedt, R. M., and D. F. Brown.** 1973. *Vibrio parahaemolyticus*: infection or toxicosis? J. Milk Food Technol. **36**:129–134.
27. **Watkins, W. D., and V. J. Cabelli.** 1985. Effect of fecal pollution on *Vibrio parahaemolyticus* densities in an estuarine environment. Appl. Environ. Microbiol. **49**:1307–1313.