Journal of Coastal Research	6	4	943-948	Fort Lauderdale, Florida	Fall 1990

Potentially Pathogenic Marine Vibrio Species in Seawater and Marine Animals in the Sarasota, Florida, Area

John D. Buck

Department of Marine Sciences and
Institute of Marine Sciences
The University of Connecticut
Noank, CT 06340, USA

ABSTRACT

BUCK, J.D., 1990. Potentially Pathogenic Marine Vibrio Species in Seawater and Marine Animals in the Sarasota, Florida, Area. *Journal of Coastal Research*, 6(4), 943–948, Fort Lauderdale, Florida, ISSN 0749-0208.

Potentially human-pathogenic *Vibrio* species were isolated from Gulf of Mexico and Sarasota Bay waters. *Vibrio alginolyticus, V. damsela, V. fluvialis,* and *V. parahaemolyticus* were recovered by agar plating and/or a most probable number technique. The same species were found associated with a variety of teleost fish, elasmobranchs, shrimp, molluscan shellfish, and bird droppings. Because Sarasota Bay may be used more intensively for commercial and recreational purposes in the future, these preliminary observations will be useful for subsequent studies of the bay under the National Estuary Program. The increasing occurrence of gastrointestinal and wound infections from marine *Vibrio* spp. suggests that these bacteria should be included in the assessment of general water quality.

ADDITIONAL INDEX WORDS: *Sarasota Bay, water quality, National Estuary Program, agar plating, marine bacteria, shellfish, human pathogens.*

INTRODUCTION

Marine bacteria in the genus *Vibrio* have emerged as serious human pathogens (TISON and KELLY, 1984; JANDA *et al.*, 1988) via consumption of raw shellfish or puncture-type wounds involving seawater; 11 species are now recognized as clinically significant. Because the northern gulf coast of Florida supports a substantial commercial oyster fishery, information exists on the association of vibrios with these molluscs (MADDEN *et al.*, 1982; DePAOLA *et al.*, 1983; RODRICK *et al.*, 1984) and some literature is available on the presence of vibrios in Gulf waters (PETERSON and YOKEL, 1983; DePAOLA *et al.*, 1984; HOOD *et al.*, 1983). Although species of *Vibrio* have been reported in sharks in the Gulf of Mexico near Sarasota (BUCK, 1984), the only other citation on the occurrence of these bacteria in this area of Florida concerns *V. alginolyticus* densities (BUCK and PIERCE, 1989). Sarasota Bay has recently been included in the National Estuary Program by the Environmental Protection Agency and the bay may be used more extensively as a commercial and recreational resource (ESTEVEZ, 1989). Consequently, it is important to begin to determine water quality in this locale. The present study, conducted between Jan. and June of 1989, is a preliminary report of the occurrence of potentially pathogenic vibrios in waters near Sarasota and the association of these bacteria with a variety of marine animals in the area.

89041 received 31 July 1989; accepted in revision 23 May 1990.

METHODS

Water

Subsurface (*ca.* 10 cm) samples were collected in sterile polyethylene bottles. Samples were taken from the Gulf of Mexico up to 15 km offshore west and northwest of Sarasota (27° 20′ N; 82° 40′ W), in Sarasota Bay, and in New Pass which separates the two bodies of water at the southern end of Longboat Key. Dilutions were prepared in sterile seawater and plate counts for heterotrophic bacteria were made on Marine Agar 2216 (MA; Difco Laboratories, Detroit, Michigan) by the spread plate method; incubation was for three to five days at 25°C. Repre-

sentative colonies were selected from all plates and maintained on MA slants. Isolates showing characteristics of the genus *Vibrio* (Gram negative, cytochrome oxidase positive, susceptibility to the vibriostatic agent 0/129, typical growth on thiosulfate citrate bile salts sucrose agar [TCBS; Difco]) were identified to species using the API 20E system (Analytab Products; Plainview, New York) with 20‰ artificial seawater as diluent. In some cases, additional tests (*e.g.*, growth at varying NaCl concentrations) were used to confirm species assignment.

On one day, two plates of MA were exposed to the air near the shore on Longboat Key. The plates were left open in a horizontal position for five min. at a distance of approximately 30 m from the Gulf of Mexico and at a height of about two m above sea level. Plates were incubated at 25°C for three days and colonies identified as above.

For quantitative determination of vibrio densities, a nine-tube most probable number (MPN) procedure was used. Ten, 1.0, and 0.1 ml volumes of water were incubated for 18–24 hr. at 37°C in 10 ml volumes of alkaline peptone broth (APB) which contained 1% Peptone (Difco) and 1% NaCl; pH was adjusted to 8.5. Three tubes for each inoculum volume were used; APB was double-strength for 10 ml inocula. Plates of TCBS agar were streaked from tubes showing turbidity and incubated at 37°C for 24 hr. Characteristic colonies were selected and identified as *Vibrio* spp. MPN/100 ml of individual species was calculated from appropriate MPN tables.

Molluscs

Samples of southern quahogs (*Mercenaria campechiensis*) and oysters (*Crassostrea virginica*) were collected from Sarasota Bay. Two collections of each species were considered and were taken from two different areas. In each case, six animals were used as a sample. Clams were opened aseptically and the contents of each (meat and liquor) transferred to a sterile blender and mixed for 30 sec. A 0.5 ml portion of each homogenate was inoculated into APB broth and incubated at 18–24 hr. at 37°C.

Fish and Shrimp

All fish were caught in the Gulf of Mexico within 15 km of Sarasota or in Sarasota Bay. Some were caught by hook and line (black drum, flounder, pigfish, catfish, squirrelfish, stingray). Other teleosts and small sharks were caught in gill nets; larger sharks were taken by long line fishing. Some fish were kept in outdoor tanks which contained circulating Sarasota Bay water. All fish were examined for bacteria while still alive. Sterile polyester-tipped swabs were used to sample gills, mouth, spines, and teeth. The intestinal tract was examined by aseptically dissecting the fish and swabbing material from the lower tract. Tips of the swabs were broken off in tubes of APB broth and incubated for 18–24 hr. at 37°C.

Shrimp were obtained on different occasions from three local bait shops on Sarasota Bay. Water from several shrimp was drained on paper towels and the whole animals were placed in sterile seawater and shaken gently. One-half ml of this rinse was inoculated into APB and incubated as above. In some cases, sterile #2 longshank stainless steel fishhooks were passed through the head or abdominal segments simulating the use of shrimp as bait for recreational fishing. The hooks were then removed by reversing the procedure and the hooks were placed in APB and incubated. Also, the intestinal tract from several shrimp was dissected out and incubated in APB.

Mysids were laboratory-reared for bioassay purposes and kept in small aquarium-type tanks containing filtered Sarasota Bay water:dechlorinated tap water (1:1). Several mysids approximately 1 cm long were drained as above, rinsed, and introduced into APB broth.

All APB tubes showing turbidity after 18–24 hr. incubation were streaked on TCBS agar. Characteristic *Vibrio* colonies were subcultured on MA slants and identified to species as described previously.

RESULTS AND DISCUSSION

Water

Of 18 water samples where quantitative platings for heterotrophic bacteria were made, 12 samples showed the presence of *Vibrio* species in 0.1 ml volumes of water. *Vibrio alginolyticus* was recovered most frequently (10 samples) and *V. fluvialis* was isolated from two samples. Of the six samples where no vibrios were found on

plates, three included platings where dilutions of only greater than 10^{-1} (<0.1 ml of water) were plated. In the remaining three samples, no vibrios were isolated on platings of 10^{-1} (0.1 ml).

Vibrio alginolyticus was recovered from both plates exposed to the air near gulf water.

These data indicated that vibrios were present in most waters sampled. Table 1 presents quantitative observations in the form of MPN estimates which substantiated the notion above that vibrios were not present in small (<0.1 ml) volumes of seawater. In only two samples (one tube each), APB inoculated with 0.1 ml of water showed subsequent evidence of *Vibrio* growth.

JANDA *et al.* (1988) summarize previous data on vibrio MPNs. Only *V. cholerae* values are available for Florida shellfish; no information for water is provided except for one earlier brief study (BUCK and PIERCE, 1989) which involved one water sample from Florida and two from North Carolina. The quantitative data in Table 1 are additional values for Gulf of Mexico and Sarasota Bay waters and are the first MPN estimates of *V. damsela* in any U.S. coastal water. The density noted here for *V. parahaemolyticus* (2,400/100 ml) is an order of magnitude higher than that reported for this organism in waters of Chesapeake Bay and Louisiana (JANDA *et al.*, 1988). It should be noted that the data herein were gathered during the coolest annual temperature in the sampling area. Abundance of marine vibrios is associated with water temperature with greatest numbers recovered at higher water temperatures. It might be expected that waters in the gulf and bay would show increased densities of the three species reported, as well as others, during the warmer months. The particularly dangerous *V. vulnificus* (BLAKE *et al.*, 1979; HOWARD *et al.*, 1986) seems to be restricted to higher temperatures (>20°C) and salinities (>16‰) (KELLY, 1982; TAMPLIN *et al.*, 1982) and may account for its absence in the Sarasota area because one or both of these parameters may have been restrictive during the study period. The same factors may explain the absence of *V. cholerae* which is essentially an estuarine organism (HOOD *et al.*, 1983).

Molluscs

Vibrio alginolyticus and *V. parahaemolyticus* were recovered from one sample of oysters (six individuals). The former was found in one quahog sample. Although the total number of samples (four) was small, these observations confirm the common occurrence of vibrios in molluscan shellfish (RODRICK *et al.*, 1984).

Fish and Shrimp

Table 2 shows the occurrence of vibrios in teleost fish, elasmobranchs, and shrimp. The three *Vibrio* spp. recovered from water were also common here; *V. fluvialis* was found in only one sample of shrimp. Because fish and shrimp are valuable resources from both commercial and recreational standpoints, it is important to recognize that many human infections have been caused by ingesting or coming in contact with these marine animals (see *e.g.*, HOWARD *et al.*, 1986 and JANDA *et al.*, 1988 for clinical histories). Persons who fish and/or handle fish

Table 1. *Most probable number (MPN) of* Vibrio spp. *in water.*

Sampling site[1]	Organism	MPN/100 ml
SB; off City Island	*Vibrio alginolyticus*	2,400
	V. parahaemolyticus	4,600
SB; N end of Longboat Key	*V. alginolyticus*	240
NP; E of bridge	*V. alginolyticus*	240
NP bridge	*V. alginolyticus*	240
NP; sea buoy	*V. alginolyticus*	23
GM; 7 km W of NP	*V. alginolyticus*	23
GM; 6 km W of NP	*V. alginolyticus*	240
GM; 2 km W of NP	*V. alginolyticus*	23
GM; off Siesta Key	*V. alginolyticus*	23
	V. damsela	240
GM; Off Longboat Key	*V. alginolyticus*	23
	V. damsela	460

[1]GM = Gulf of Mexico; NP = New Pass; SB = Sarasota Bay

Table 2. *Vibrio spp. isolated from fish and shrimp.*

Common name	Scientific name	Source[1]	Site of isolation of *Vibrio* spp.[2] V. a.[3]	V. p.	V. d.	V. f.
Yellow jack	*Caranx bartholomaei*	T	i, t	g		
Black drum	*Pogonias cromis*	GM, SB	g, i, m, sp			
Black sea bass	*Centropristis striata*	T	i, sp	sp, t		
Nassau grouper	*Epinephelus striatus*	T	i, sp, t	sp		
Mullet	*Mugel cephalus*	SB	i, m			
Spanish mackerel	*Scomberomorus maculatus*	GM	i, sp, t	sp		
Pompano	*Trachinotus carolinus*	GM	g, i, sp, t	g		
Flounder	*Pseudopleuronectes americanus*	GM	g, i, m, sp	t		
Pigfish	*Orthopristis chrysoptera*	GM	sp			
Catfish	*Arius felis*	SB	i, sp	sp		
Squirrelfish	*Holocentrus rufus*	GM			sp, t	
Sandbar shark	*Carcharinus plumbeus*	GM	t	t		
Lemon shark	*Negaprion brevirostris*	GM	t	t		
Blacktip shark	*Carcharinus limbatus*	GM	t	t		
Spinner shark	*Carcharinus brevipinna*	GM	t			
Bull shark	*Carcharinus leucas*	GM	t			
Nurse shark	*Ginglymostoma cirratum*	T	g, i, t			
Clearnose skate	*Raja eglanteria*	T	t			
Southern stingray	*Dasyatis americana*	SB	sp, t			
Shrimp	*Penaeus* spp.	BS 1	s	s		
		BS 2	s			i
		BS 3	s	s		
Mysid	*Mysidopsis bahia*	T	s			

[1]BS = bait shop; GM = Gulf of Mexico; SB = Sarasota Bay; T = lab tank
[2]V. a. = *V. alginolyticus*; V. d. = *V. damsela*; V. f. = *V. fluvialis*; V. p. = *V. parahaemolyticus*
[3]g = gills; i = intestinal tract; m = mouth; s = surface; sp = spines; t = teeth

touch gills and intestinal contents and skin may be punctured by teeth or spines. While healthy individuals are generally not susceptible to *Vibrio* infections, there are high risk groups; persons with liver disease and/or immunosuppression are particularly vulnerable (TILTON and RYAN, 1987).

The occurrence of potentially pathogenic vivrios on the teeth of all six species of sharks sampled confirms previous studies (BUCK, 1984; BUCK *et al.*, 1984) and reinforces that caution should be exercised in handling these animals to avoid lacerations which may become infected.

Similarly, the transfer of *Vibrio* spp. from the surface of bait shrimp to fish hooks emphasizes that recreational fishing may pose a potential hazard via cuts. A 19 year old male with no predisposing factors contracted a *V. alginolyticus* infection from a fish hook puncture (HOWARD *et al.*, 1986).

In a study to be reported elsewhere, 80% of fresh gull (*Larus argentatus, L. delawarensis, L. atricilla*) and 42% of pelican (*Pelecanus occidentalis*) droppings collected in the Sarasota Bay area contained the five *Vibrio* spp. noted above in water, fish, shrimp, and shellfish. Because these birds are ubiquitous in subtropical coastal areas of Florida, they may represent a significant reservoir of vibrios (and other pathogens) in areas where fishing and fish processing are concentrated.

Blooms of the toxic dinoflagellate *Ptychodiscus brevis* ("red tide") occur frequently in the Sarasota area. Because marine *Vibrio* species, including *V. alginolyticus* and *V. parahaemolyticus*, produce the strong neurotoxin tetrodotoxin or its derivatives (SIMIDU *et al.*, 1987), it may be necessary to consider these bacteria in the overall ecology of the red tide phenomenon; *e.g.*, are autochthonous vibrio populations stimulated by dinoflagellate blooms? *Vibrio alginolyticus* was recovered from both plates exposed to air near the gulf. If vibrio populations increase during red tides, large numbers of bacteria may be transported onshore via aerosols. This study provides an additional data base on vibrio densities during nonbloom conditions. BUCK and PIERCE (1989) reported an MPN value of > 1,100/100 ml of *V. alginolyticus* for one Gulf of Mexico nonbloom water sample in May 1988.

CONCLUSIONS

Potentially pathogenic marine *Vibrio* species were isolated frequently from waters of the Gulf of Mexico near Sarasota, from Sarasota Bay, and from a variety of marine animals. The presence of these bacteria should be recognized by both medical authorities and those concerned with the commercial and recreational development of Sarasota Bay. Because the occurrence of marine-acquired *Vibrio* infections is being increasingly reported, shoreline medical facilities should be alert to the role of marine bacteria in both gastrointestinal and wound infections, particularly in high risk groups. Clinical laboratories may not culture for vibrios and thus disease may remain undiagnosed and incidence underreported (TILTON and RYAN, 1987).

Increased use of the bay in the future, especially for shellfishing, should include an assessment of vibrios in species consumed raw; *i.e.*, clams and oysters. Raw consumption of these shellfish is historically the major cause of vibrio disease. Of particular interest is the conclusion that vibrio densities do not correlate well with traditional pollution indicators such as coliforms (HOOD *et al.*, 1983; DePAOLA *et al.*, 1983; RODRICK *et al.*, 1984). Consequently, special monitoring and standard establishment may be appropriate in the future. It should be noted that *Vibrio cholerae* (and other human pathogens) exist in the environment in a viable but nonculturable state (COLWELL *et al.*, 1985) and thus are probably underestimated by current methods. This reinforces the need for wider recognition of this phenomenon and the importance of developing more efficient recovery techniques.

Caution is also urged in commercial and recreational fishing because vibrios occur on internal and external surfaces of fish and shrimp caught in the area. Lacerations from tooth bites and spine and hook punctures are particularly involved.

Preliminary data suggest that gulls, pelicans, and probably other aquatic birds may serve as reservoirs to spread vibrios over larger coastal and inland areas.

ACKNOWLEDGEMENTS

This research was conducted while on sabbatic leave at the Mote Marine Laboratory, Sarasota, Florida. I am indebted to Dr. Kumar Mahadevan, Director, and Dr. Richard H. Pierce, Associate Director, for providing space and facilities. Thanks are due Dr. Carl Luer, Dr. Robert Heuter, Capt. Peter Hull, Capt. Mike Bacon, Jeff Bauer, Niell Faulman, Frank Gangi, Carmen Harris, Patti Hasbrouck, and Jason Weeks for aid in collecting samples. The research was supported in part by a pilot grant from the Center for Environmental Health, The University of Connecticut. This is contribution No. 220 from The University of Connecticut, Institute of Marine Sciences.

LITERATURE CITED

BLAKE, P.A.; MERSON, M.H.; WEAVER, R.E.; HOLLIS, D.D., and HEUBLEIN, P.C., 1979. Disease caused by a marine vibrio: clinical characteristics and epidemiology. *New England Journal of Medicine*, 300, 1–5.

BUCK, J.D., 1984. Quantitative and qualitative bacteriology of elasmobranch fish from the Gulf of Mexico. *Fishery Bulletin*, 82, 375–381.

BUCK, J.D., and PIERCE, R.H., 1989. Bacteriological aspects of Florida red tides: A revisit and newer observations. *Estuarine, Coastal and Shelf Science* (in press).

BUCK, J.D.; SPOTTE, S., and GADBAW, J.J., Jr., 1984. Bacteriology of the teeth from a great white shark: Potential medical implications for shark-bite victims. *Journal of Clinical Microbiology*, 20, 849–851.

COLWELL, R.R.; BRAYTON, P.R.; GRIMES, D.J.; ROSZAK, D.B.; HUQ, S.A., and PALMER, L.M., 1985. Viable but non-culturable *Vibrio cholerae* and related pathogens in the environment: Implications for release of genetically engineered microorganisms. *Bio/Technology*, 3, 817–820.

DePAOLA, A.; PRESNELL, M.W.; MOTES, M.L., Jr.; McPHERSON, R.M.; TWEDT, R.M.; BECKER, R.E., and ZYWNO, S., 1983. Non-O1 *Vibrio cholerae* in shellfish, sediment and waters of the U.S. Gulf coast. *Journal of Food Protection*, 46, 802–806.

DePAOLA, A.; PRESNELL, M.W.; BECKER, R.E.; MOTES, M.L., Jr.; ZYWNO, S.R.; MUSSELMAN, J.F.; TAYLOR, J., and WILLIAMS, L., 1984. Distribution of *Vibrio cholerae* in the Apalachicola (Florida) Bay estuary. *Journal of Food Protection*, 47, 549–553.

ESTEVEZ, E.F., (ed.), 1989. *Tampa and Sarasota Bays: Issues, Resources, Status, and Management.* Washington, D.C.: NOAA Estuarine Programs Office, Seminar Series No. 11, 215p.

HOOD, M.A.; NESS, G.E.; RODRICK, G.E., and BLAKE, N.J., 1983. Distribution of *Vibrio cholerae* in two Florida estuaries. *Microbial Ecology*, 9, 65–75.

HOWARD, R.; BRENNAMAN, B., and LIEB, S., 1986. Soft tissue infections in Florida due to marine

Vibrio bacteria. *Journal of the Florida Medical Association*, 73, 29–34.

JANDA, J.M.; POWERS, C.; BRYANT, R.G., and ABBOTT, S.L., 1988. Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. *Clinical Microbiology Reviews*, 1, 245–267.

KELLY, M.T., 1982. Effect of temperature and salinity on *Vibrio (Beneckea) vulnificus* in a gulf coast environment. *Applied and Environmental Microbiology*, 44, 820–824.

MADDEN, J.M.; McCARDELL, B.A., and READ, R.B., Jr., 1982. *Vibrio cholerae* in shellfish from U.S. coastal waters. *Food Technology*, March, 93–96.

PETERSON, M.E., and YOKEL, B.J., 1983. Recovery of pathogens from Naples Bay, Florida. *Abstracts of the Annual Meeting, American Society for Microbiology*, p. 278.

RODRICK, G.E.; BLAKE, N.J.; TAMPLIN, M.; CORNETTE, J.E.; CUBA, T., and HOOD, M.A., 1984. The relationship between fecal coliform levels and the occurrence of vibrios in Apalachicola Bay, Florida. *In*: Colwell, R.R., (ed.), *Vibrios in the environment*. New York: Wiley, pp. 567–575.

SIMIDU, U.; NOGUCHI, T.; HWANG, D.; SHIDA, Y., and HASHIMOTO, K., 1987. Marine bacteria which produce tetrodotoxin. *Applied and Environmental Microbiology*, 53, 1714–1715.

TAMPLIN, M.; RODRICK, G.E.; BLAKE, N.J., and CUBA, T., 1982. Isolation and characterization of *Vibrio vulnificus* from two Florida estuaries. *Applied and Environmental Microbiology*, 44, 1466–1470.

TILTON, R.C., and RYAN, R.W., 1987. Clinical and ecological characteristics of *Vibrio vulnificus* in the northeastern United States. *Diagnostic Microbiology and Infectious Disease*, 6, 109–117.

TISON, D.L., and KELLY, M.T., 1984. Vibrio species of medical importance. *Diagnostic Microbiology and Infectious Disease*, 2, 263–276.