FRI Briefings

Non-Cholera Vibrios

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Oyster beds in Galveston Bay, Texas, were closed to harvesting during most of the summer of 1998 because 416 persons in 13 states fell ill after eating raw oysters traced to this location (*21*). Then, just as these shellfish beds appeared to be safe again, New York authorities forbade the harvest of clams and oysters from beds near Long Island in mid-September, after 10 people became ill from eating raw shellfish collected there. The bacterial pathogen contaminating shellfish in both areas was identified as *Vibrio parahaemolyticus*. In the summer of 1997, this organism caused another large outbreak in the Pacific Northwest with 209 cases and one death (*8*). A related species, *V. vulnificus*, was blamed for two fatalities in Florida in 1998 and for a total of 33 deaths in the U.S. in 1996 (*9,20*).

What kind of bacteria are these vibrios and where do they come from?

Vibrios are short, Gram-negative rods, usually found in aquatic environments. Some, such as *V. vulnificus* and *V. parahaemolyticus* are halophiles which require a saltwater environment for growth. Both species are normal residents in coastal waters, and their numbers depend on the temperature and salinity of the water (*12,15,17*). Cell numbers typically increase during the summer as water temperatures rise but they are not positively correlated with indicators of fecal contamination. Therefore, vibrio concentrations are not necessarily higher in shellfish from beds closed to harvesting because of high coliform counts or evidence of sewage contamination. Numerous surveys of raw shellfish from coastal waters of the U.S. have demonstrated a high level of contamination with vibrios. One survey, conducted in the summer, indicated that 100% of the oysters tested contained detectable *V. parahaemolyticus* and 67% contained *V. vulnificus*. Not all strains of these vibrio species are pathogenic. But as yet we don't know if certain environmental conditions favor the growth of pathogens over non-pathogens (*23*).

In the U.S., *V. parahaemolyticus* and *V. vulnificus* are the most common vibrios identified in seafood associated with illness (*9*). However, as many as 8 species of *Vibrio* have been implicated in cases and outbreaks of foodborne disease. With the exception of cholera, *Vibrio* diseases are not designated as reportable. However, the CDC's foodborne disease surveillance program (FoodNet) does monitor vibrio infections at its five target locations. In 1997, FoodNet reported that vibrios were isolated from 51 cases of foodborne illness, including 31 in California, 12 in Oregon, four in Connecticut, and two each in Georgia and Minnesota (*27*). Probably the best known pathogen in this genus, worldwide, is *V. cholerae* which causes large epidemics of cholera in developing nations, particularly where sanitation is inadequate. Seafood is an important vehicle for cholera but fecal contamination of drinking water and food is often responsible for the widespread and persistent nature of cholera outbreaks (*19,23*). *Vibrio cholerae* survives in salt

water but is not an obligate halophile; therefore it can also grow and reproduce in freshwater environments.

Vibrio vulnificus

Although *V. vulnificus* and *V. parahaemolyticus* live in similar environments and may be amenable to similar control strategies, they cause quite different types of foodborne illness. *Vibrio vulnificus* does not cause large outbreaks but rather, severe and often fatal infections in susceptible individuals who consume as few as 100 bacterial cells (*5,14,23*). Healthy persons with an intact immune system and a well-functioning liver can apparently consume raw oysters containing *V. vulnificus* without becoming sick at all or with only a mild case of gastroenteritis. Persons at high risk for infection include those with liver disease, cancer, AIDS, chronic kidney disease, diabetes, and inflammatory bowel disease. In fact, liver disease emerged as the primary risk factor for a series of *V. vulnificus* cases reported during 1988–1996: 80% of the 120 fatal cases for whom data were available had liver disease, primarily cirrhosis or hepatitis (*28*).

Susceptible individuals may also be infected by exposure to brackish or salt water containing *V. vulnificus* if the skin is broken. Of the Florida cases occurring this past summer, one person was infected when a blue crab scratched his hand while another person had a cut on his foot which was exposed to sea water (*20*). Approximately 48% of the 422 cases reported in 1988–1996 followed ingestion of raw or lightly cooked seafood while 45% were due to wound infections; many of the latter were associated with occupational exposures (*28*).

Reduced stomach acidity and an impaired immune system allow *V.vulnificus*, ingested in raw seafood, to cross the intestinal mucosa, enter the blood stream, and cause primary septicemia. Symptoms develop from 7 hours to several days after exposure. Fever, chills, nausea, and hypotension are often followed by necrotizing fascitis (severe skin lesions) on the extremities. For those who develop septicemia, only 40–60% survive even with appropriate treatment.

Vibrio vulnificus strains are known to vary in virulence. Pathogenic isolates produce an acidic capsular polysaccharide which protects the cells from phagocytosis and other antibacterial responses of the immune system. In addition, this polysaccharide stimulates the release of tumor necrosis factor a and other cytokines from peripheral white blood cells. This overproduction and dysregulation of inflammatory cytokines causes septic shock and death in many patients. Other virulence factors may be involved in causing illness but they have not been characterized as yet. Studies are underway to develop methods to distinguish between pathogenic and non-pathogenic strains (*25,26*).

Vibrio parahaemolyticus

In contrast, infection with *V. parahaemolyticus* generally results in a self-limiting gastroenteritis with symptoms of nausea, diarrhea, and vomiting appearing 12–24 hours after consumption of contaminated seafood (*4,23*). Following ingestion of an infective dose of *V. parahaemolyticus*, estimated at one million cells, most people will develop symptoms but will recover in 2–3 days. Some persons with underlying illness experience a more severe disease: 2 of 209 patients in the 1997 outbreak required hospitalization and one, with *Vibrio parahaemolyticus* present in the bloodstream, died. Most pathogenic isolates produce a hemolysin and some other enzymes which are involved in establishing an intestinal infection and causing diarrhea (*1,8*).

Vibrio parahaemolyticus is a common pathogen in Asia causing 197 outbreaks and 8967 cases in Taiwan during 1986–1995 as well as numerous outbreaks in Japan and some Southeast Asian countries (*22,24*). One of the disturbing aspects of this past summer's outbreak in the U.S. was the fact that the particular *V. parahaemolyticus* strain isolated from patients, O3:K6, had not previously been detected in coastal waters of the U.S. However, this virulent strain has been known in Asia since 1994 and has been increasingly isolated from cases of diarrhea in Japan, India, and other countries. This raises the possibility that this strain was introduced to U.S. coastal waters by ballast water discharged from ships which had traveled to Asia. A similar situation apparently occurred 4 years ago when *V. cholerae* was detected in oysters in Mobile Bay, Alabama. This contamination was traced to water dumped from ships which had been to Peru which was experiencing a major outbreak of cholera at the time (*21*). Preliminary reports indicate that the *V. parahaemolyticus* isolated from the Long Island oysters in September was also strain O3:K6.

Prevention of foodborne illness

Fortunately, most Americans prefer to eat their seafood cooked—baked, fried, or boiled—and this heat treatment effectively kills foodborne pathogens present in raw fish and shellfish. Vibrios are heat sensitive and destroyed by adequate cooking. However, a mild heat treatment, just sufficient to open the shells, may not bring the interior of the shellfish to a high enough temperature (140°F) to destroy vibrios concentrated there (*13*). Nevertheless, even adequate cooking cannot prevent foodborne illness if cooked shellfish are subsequently recontaminated. This scenario occurred in Louisiana in 1978 when cooked shrimp were returned to boxes in which raw shrimp had been shipped and then were held at warm temperatures for several hours before serving. *Vibrio parahaemolyticus* multiplied in the shrimp and caused 1133 cases of illness (*23*).

Precautions should also be taken to prevent an increase in numbers of vibrios between harvest and consumption. Typically, shellfish harvested in Gulf Coast waters are stored on the deck at ambient temperatures (as high as 33°C in July) until the ship returns to shore. Some data from summer months indicate that freshly harvested oysters contain a median of 2300 MPN (most probable number) of *V. vulnificus* per gram of oyster at harvest and a median of >110,000 MPN/g when the oysters arrive at the processing plant. Refrigeration of freshly harvested shellfish would prevent this increase in numbers of vibrios and also decrease the viability of the cells already present (*6,14*).

Some hardy coastal residents prefer their oysters and clams "on the half shell" (uncooked) and others in this country, and particularly in Japan, enjoy the taste of raw fish. Such inadequately cooked fish and shellfish have caused a variety of foodborne disease outbreaks due to parasitic worms, viruses, and bacteria. Since raw oysters are a significant source of a potentially lethal infection and to persons with liver disease, there are regulations in California, Florida, and Louisiana for posting warnings in places that sell raw oysters. However, investigations in California revealed that these warnings are often not present on restaurant menus. Furthermore, the warnings are usually printed only in English. The three fatalities associated with consumption of raw oysters in Los Angeles in 1996 were Hispanic males fluent in Spanish but not English. Although people with liver disease should be warned by their physicians, these men did not have health insurance and therefore did not have much contact with the health care establishment. Further outreach educational efforts should target susceptible populations (*18*).

For some pathogens concentrated by shellfish, a period of depuration (holding of shellfish in clean water for several days) can remove most of the pathogens. However, *Vibrio* spp. adhere strongly to the shellfish digestive tract and cannot be successfully removed by rinsing the shellfish or by depuration. Another procedure, called relaying, which involves suspending shellfish in water with a higher salinity at an offshore location, was found to significantly reduce numbers of *V. vulnificus*. This species is sensitive to high salt concentrations (>28 ppt, parts per thousand) and exposure of oysters for 7–17 days to water with a salinity of 30–34 ppt reduced bacterial numbers from 1,000–10,000 MPN/g to <10 MPN/g (*16*).

Since vibrios are naturally present in coastal waters and in most shellfish populations burgeon during warm weather, monitoring of water temperatures may provide an early warning of likely problems with *Vibrio* spp. Shellfish could then be analyzed for the presence of pathogenic vibrios. Traditionally vibrios were enumerated on TCBS (thiosulfate–citrate–bile salts–sucrose) agar after growth in an enrichment broth containing cellobiose or alkaline peptone with 1% sodium chloride (*10,11,23*). More recently several procedures have been developed for detection of specific pathogenic *Vibrio* spp. based on detection of DNA coding for factors associated with virulence. Gene probes specific for the cytotoxin hemolysin produced by *V. vulnificus* and the thermostable hemolysin produced by *V. parahaemolyticus* have been successfully employed in identifying these two species in shellfish (*2,7,29*). A multiplex PCR (polymerase chain reaction) system which can amplify genes specific for *E. coli*, *Salmonella typhimurium*, *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus* has also been developed for the simultaneous detection of all five pathogens (*3*).

In summary, it is impossible to eliminate pathogenic vibrios from coastal environments or from seafood as it is harvested by controlling pollution (although such measures will reduce contamination with enteric bacteria and viruses) because these bacteria are normal constituents of the environment. However, there are some ways to reduce pathogen numbers and prevent their growth in seafood intended for human consumption. These procedures—refrigeration, relaying, cooking, irradiation, and harvest during cooler months—along with good food-handling practices can minimize *Vibrio* spp. in seafood. Prominent warnings emphasizing the frequently lethal effects of *V. vulnificus* to persons with liver disease and immune dysfunction should alert those who insist on eating raw oysters.

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