

Coastal Vibrios: Identifying Relationships between Environmental Condition and Human Disease*

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ABSTRACT

Vibrio spp. cause frank and opportunistic infections of humans through exposure to seafood and seawater. Due to their natural occurrence in coastal environments, traditional indicator organisms, such as *E. coli*, do not predict their presence. This problem has complicated public health initiatives aimed at reducing the impact of illnesses from *Vibrio* spp. In the U.S., *V. vulnificus* has received extensive study due to the severity of its disease in humans. Its numbers increase with warmer summer temperature, and decline to nondetectable levels in colder winter months. In environments with salinities greater than 20 ppt, *V. vulnificus* numbers decline to levels that do not pose human health risks. A similar response to temperature has been observed for pathogenic strains of *V. parahaemolyticus*, where recent outbreaks of illness have been associated with El Niño weather conditions. In addition, temperature-induced plankton blooms have been linked to epidemic cholera in certain geographical regions of the world. New research shows that seawater temperature and salinity can be used to develop mathematical models of *V. vulnificus* incidence in coastal environments. Similar efforts might be extended to other *Vibrio* spp. to develop indicators that predict human health risk, as well as ecosystem integrity.

Key Words: mathematical models, salinity, temperature, *Vibrio vulnificus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*.

INTRODUCTION

Bacteria of the genus *Vibrio* are indigenous to coastal and marine environments, from which they occasionally cause frank and opportunistic human infections (Hlady and Klontz 1996; Fyfe *et al.* 1998). Worldwide, the notable pathogenic *Vibrio*

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spp. include *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. In the past ten years, an increasing number of infections from *Vibrio* spp. have been recognized in the U.S., resulting in initiatives to reduce their impact on human health (Ross *et al.* 1994; Hlady and Klontz 1996; Bag *et al.* 1999; Basu *et al.* 2000). These efforts have mostly involved educational interventions that should also be complemented with tools to predict their occurrence, levels, and associated risks, in U.S. coastal environments and edible seafood.

Two environmental conditions have been shown to influence levels of *Vibrio* spp. in coastal environments. First, temperature has a marked influence on the incidence of vibrios (Motes *et al.* 1998; Tamplin 1994; Kelly 1982). For example, *V. vulnificus* is not normally detected in Gulf of Mexico coastal environments during cold periods, but it can be present at levels greater than 100,000 colony-forming units per gram of oyster meat and sediment in warm summer months (Vanoy *et al.* 1992). This seasonal ecology correlates with the periodicity of human vibrio infections, indicating that risk is related to dose, and/or to the seasonal emergence of virulent strains (Jackson *et al.* 1997; Hlady and Klontz 1996).

A similar response to temperature is observed for pathogenic strains of *V. parahaemolyticus* and *V. cholerae* (Jesudason *et al.* 2000; DePaola *et al.* 1990; Huq *et al.* 1984). In the past four years, an increased number of *V. parahaemolyticus* outbreaks have been recorded worldwide, and hypothetically linked with El Niño-induced changes in surface seawater temperature (Wechsler *et al.* 1999; Fyfe *et al.* 1998). Also, outbreaks of cholera have been linked to environments with warm water temperature, high organic load, and plankton blooms (Jesudason *et al.* 2000; Lobitz *et al.* 2000).

In addition to temperature, seawater salinity exerts a strong influence on the survival of *Vibrio* spp. Nearly all *Vibrio* spp. require the sodium ion for growth, with variations observed for individual species (Elliot *et al.* 1995; Singleton *et al.* 1982). For example, *V. cholerae* requires trace levels of sodium, survives for long periods of time in fresh and brackish water, and is rarely isolated from open ocean water (Williams *et al.* 1998; Singleton *et al.* 1982). Researchers suggest that these characteristics contribute to cholera epidemics by enriching fresh water environments with high levels of infectious *V. cholerae* (Borroto 1998; Mahasneh and Al-Sayed 1997; West 1989). In contrast, most *Vibrio* spp., such as *V. vulnificus* and *V. parahaemolyticus*, do not survive in freshwater rivers and lakes, but are able to grow at the salinities found in coastal and open ocean waters (Mahasneh and Al-Sayed 1997; Kaspar and Tamplin 1993; Chowdhury *et al.* 1990; DePaola *et al.* 1990; Kelly 1982).

Historically, it has been difficult to develop indicators for *Vibrio* spp. due to their ubiquitous occurrence in coastal waters and the lack of physiological characteristics shared with human enteric bacteria (Rodrick *et al.* 1984; Tamplin *et al.* 1983; Tamplin *et al.* 1982). Common indicator organisms for human gastroenteritis, such as *E. coli*, cannot predict the presence of *Vibrio* spp. in water or food. This has complicated public health measures aimed at reducing the impact of vibrio disease. However, recent research indicates that seawater temperature and salinity may be used to predict the occurrence of *Vibrio* spp., which could be useful for estimating health risks (Motes *et al.* 1998; Tamplin *et al.* 1996).

The following report addresses the epidemiology, pathology, and ecology of *V. vulnificus*, a coastal bacterium that has impacted human health and public health

policy in the US, and to a lesser degree, *V. parahaemolyticus* and *V. cholerae*.

HUMAN DISEASE CAUSED BY *VIBRIO VULNIFICUS*

Vibrio spp. are the leading cause of bacterial illness linked to the consumption of raw and partially cooked molluscan shellfish (Food and Nutrition Board 1991). *V. vulnificus* causes more reported disease in the US than *V. cholerae*, and is the leading cause of reported death due to the ingestion of seafood (Mead *et al.* 1999; Hlady and Klontz 1996). Its effect on regulatory policy is evidenced in various states, among them Florida, California, and Louisiana, which require consumer advisories/warnings to be posted at all points-of-sale for retail raw molluscan shellfish (MacNeil 1991). Moreover, Hawaii bans the sale of raw shellfish that contain *V. vulnificus* and are destined for human consumption.

V. vulnificus can cause fulminant and life-threatening infections in high-risk individuals (*e.g.* persons with liver disease and hemochromatosis) who consume warm water-harvested raw oysters from the Gulf of Mexico, and in persons who suffer skin wounds in estuarine environments (Kumamoto and Vukich 1998; Klontz *et al.* 1988). The consumption of molluscan shellfish presents an increased risk to human health for two primary reasons. First, shellfish filter and concentrate particles from surrounding seawater. Second, shellfish are frequently eaten raw or undercooked, thus allowing infectious microbes access to internal human tissues.

In Florida, between 1981 and 1993, there were 71 cases of *V. vulnificus* oyster-associated septicemia that required hospitalization; of these cases, 77% resulted in death (Hlady and Klontz 1996). In addition, there were 34 cases of *V. vulnificus* wound infection with a mortality rate of 15% (Hlady and Klontz 1996). A recent report of US food-related illnesses by the Centers for Disease Control and Prevention estimated that there are 97 annual *V. vulnificus* infections and 48 associated deaths each year in the US (Mead *et al.* 1999).

The hallmark of systemic disease caused by *V. vulnificus* is a fulminating infection and extensive invasion of host tissues (Kumamoto and Vukich 1998; Klontz *et al.* 1988). The major predisposing condition is tissue iron overload, classified as hemochromatosis (Kraffert and Hogan 1992; Brennt *et al.* 1991; Bullen *et al.* 1991). Primary hemochromatosis in humans is the result of mutations in the *hfe* gene resulting in increased uptake of iron by the intestines, and it is believed to be the most common autosomal recessive genetic defect in the Caucasian population (Burke *et al.* 1998); hence the susceptible population for *V. vulnificus* infection is potentially large. Secondary hemochromatosis may also be the result of liver disease, such as cirrhosis from alcoholism or hepatitis (Brennt *et al.* 1991; Bullen *et al.* 1991, Muench 1989). Additionally, people with diabetes mellitus and immunodeficiency are at risk for *V. vulnificus* disease (Kumamoto and Vukick 1998; Hlady and Klontz 1996).

Disease and death can occur in susceptible individuals as soon as 24 hours after consumption of oysters containing *V. vulnificus*, or after bringing a skin wound in contact with seawater. By the time the diagnosis is made, it is often too late for therapeutic intervention. The ability of the bacteria to rapidly invade through tissues contributes to the disease process.

The opportunistic nature of the disease is illustrated by the large numbers of *V. vulnificus* that are consumed by the general population without illness. For instance, one dozen raw "summer-time" Gulf of Mexico oysters typically contain in excess of 10 million total *V. vulnificus* (Vanoy *et al.* 1992; Tamplin *et al.* 1990). Currently, the infective dose of *V. vulnificus* for humans is not well understood, but evidence indicates that it is associated with concentrations greater than 1000 colony-forming units per gram of oyster meat (Jackson *et al.* 1997; Tamplin 1994).

ECOLOGY OF *V. VULNIFICUS*

V. vulnificus is a naturally occurring estuarine organism found in temperate and tropical waters throughout the world. It is isolated commonly from seawater, sediment, and various marine life forms (Kaysner *et al.* 1987; Oliver *et al.* 1982; Tamplin *et al.* 1983; Tamplin *et al.* 1982). It and other *Vibrio* spp. exist in seawater, on a variety of surfaces including plankton, sediment, and fish, as well as within tissues of filter-feeding molluscan shellfish.

The ecology of *V. vulnificus* in seawater is markedly affected by temperature and enhanced by salinities of 5 to 20 ppt (Motes *et al.* 1998; Tamplin 1994; Kelly 1982; Oliver *et al.* 1982; Tamplin *et al.* 1982). Our laboratory, in collaboration with health agencies in 15 coastal states, observed that *V. vulnificus* can be found in many coastal US waters, ranging from Maine to Washington (Tamplin 1994).

When salinity levels are adequate for *V. vulnificus* survival, such as in Gulf of Mexico estuaries, temperature is a primary factor that controls *V. vulnificus* growth, survival, and decline (Motes *et al.* 1998; Tamplin 1994). When water temperature is below 20°C, *V. vulnificus* numbers decline in seawater, and persist in sediment and oysters (Tamplin 1994). When water temperature declines below 15°C, it is not usually detected in environmental samples by bacteriological culture.

The effect of salinity on *V. vulnificus* survival can be observed in environments where temperature is relatively constant year-round, such as in Hawaiian coastal waters (Tamplin 1994). For example, along the shores of the Hawaiian islands, *V. vulnificus* is not found in high salinity seawater nor in freshwater beach upwellings. However, it is isolated in relatively high concentrations at the open ocean-fresh water interface. These observations are also confirmed in laboratory microcosms of defined salinity, showing that *V. vulnificus* survival is enhanced at 5 to 10 ppt, it declines to non-detectable levels at salinities greater than 20 ppt, and it lyses in fresh water (Kaspar and Tamplin 1993).

Currently, no microbiological indicator of *Vibrio* spp. exists that is analogous to the fecal coliform as an indicator of human enteric pathogens. However, there has been good progress to develop mathematical models based on quantitative measurements of seawater temperature and salinity.

DEVELOPMENT OF MICROBIAL MODELS

Two research groups have conducted extensive environmental research to define environmental factors associated with the incidence and levels of *V. vulnificus* in molluscan shellfish. Motes *et al.* (1998) investigated temperature and salinity parameters associated with US waters and oysters linked to foodborne *V. vulnificus* infec-

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tions. At sites along the Gulf of Mexico, *V. vulnificus* numbers increased with water temperature up to 26°C and were constant at higher temperatures. High (>10,000cfu/g) *V. vulnificus* levels were found in oysters from salinities of 5 to 25 ppt. Lower *V. vulnificus* numbers (< 100 cfu/g) were found at salinities greater than 28 ppt, typical of Atlantic Coast sites.

Tamplin (1994) and collaborators studied *V. vulnificus* levels in seawater, sediment and oysters collected at monthly test intervals from commercial shellfish harvesting sites in 15 coastal states that included Maine, Connecticut, Rhode Island, Massachusetts, New Jersey, Virginia, South Carolina, Florida, Mississippi, Louisiana, Texas, California, Oregon, Washington, and Hawaii. *V. vulnificus* was isolated, speciated, and enumerated using published test methodologies (Tamplin *et al.* 1990). The results of this study showed that of the two predictors (temperature and salinity), temperature had the highest correlation with *V. vulnificus* levels in oysters. A linear regression formula was derived that predicted the levels of *V. vulnificus* in Gulf of Mexico shellfish based upon seawater temperature and salinity (Tamplin *et al.*, 1996).

$$-6.32 + (0.23*\text{sal}) + (0.347*\text{temp}) - (0.0056*\text{sal}*\text{temp}) - (0.0039*\text{sal}*\text{sal}) = \log \text{ cfu/g}$$

where sal = salinity (ppt) and temp = temperature (°C)

EVIDENCE OF ENVIRONMENTAL LEVELS OF *V. VULNIFICUS* ASSOCIATED WITH DISEASE

One approach to defining infectious doses for a foodborne pathogen is to measure the quantity of the implicated species in a case-associated food. However, this approach assumes that the food is populated by a single strain, or that if multiple strains exist, all have equal virulence. It also assumes that the change in pathogen levels between the time of consumption and analysis can be estimated.

In this regard, Buchreiser *et al.* (1995) showed that individual oysters can have multiple strains of *V. vulnificus*, sometimes more than 100 per oyster, as evidenced by pulsed-field gel electrophoresis (PFGE). This finding demonstrated the challenge for retrospective studies of *V. vulnificus* infections, since the pathogenic strain(s) would need to be enumerated apart from the total *V. vulnificus* flora. This approach is supported by observations that only one PFGE profile is found in the blood of an infected individual, even though multiple strains were ingested (Jackson *et al.* 1997).

In an effort to gather evidence about environmental levels associated with human disease, total *V. vulnificus* levels were measured in oysters harvested from a commercial site in Apalachicola Bay, Florida, over 3 years (Jackson *et al.* 1997). During this study, the Florida Department of Environmental Protection monitored the incidence of human *V. vulnificus* disease associated with oysters harvested from the Apalachicola Bay location, and noted those cases where the implicated oysters were harvested within at least 72 hours of sampling time. A total of eight infections met these criteria, and it was observed that *V. vulnificus* disease occurred when *V. vulnificus* levels in oyster meat exceeded 1000 CFU/g (range 10³/g to 10⁵/g) (Jackson *et al.* 1997). This concentration translated to approximately 3 × 10⁵ *V. vulnificus* in a dozen raw oysters.

In a separate series of studies, *V. vulnificus* was enumerated in oysters associated with human infections (Jackson *et al.* 1997). Logistical constraints included identifying cases, obtaining implicated oysters from homes and retail outlets, and collecting clinical samples from patient specimens. For a total of four cases where clinical specimens were obtained, each patient displayed a single strain, as defined by PFGE. In one human infection, oysters obtained from the implicated restaurant were analyzed to determine both the concentration and genetic diversity of *V. vulnificus* isolates. The oysters contained 9.6×10^3 /g *V. vulnificus* oyster, indicating that the patient ingested approximately 6×10^5 CFU. Analysis by PFGE revealed eight unique DNA profiles among the ingested strains. The strain that infected the individual was present in the implicated oysters at 2×10^3 CFU/g meat, indicating that approximately 1×10^5 CFU were consumed.

ENVIRONMENTAL INFLUENCES ON *V. PARAHAEMOLYTICUS* AND *V. CHOLERAE* ECOLOGY AND DISEASE

V. parahaemolyticus inhabits many salt water environments. Even though its numbers increase with increasing seawater temperature, it is less restricted to warm, low salinity coastal waters as observed for *V. cholerae* and *V. vulnificus* (Jesudason *et al.* 2000). *V. parahaemolyticus* is one of the most common causes of bacterial seafood-borne disease in the U.S. (Hlady and Klontz 1996; Food and Nutrition Board 1991). Human illness is usually associated with cooked seafood that has been cross-contaminated by seawater or by uncooked seafood. The disease presents as a self-limiting form of mild to severe gastroenteritis.

Between May and September of 1997, over 250 human *V. parahaemolyticus* infections occurred in the Pacific Northwest of the U.S. associated with raw oyster consumption (Fyfe *et al.* 1998). All illnesses were caused by a strain with the O4 serotype, which also possessed the virulence-associated thermal direct hemolysin gene. At the time of the outbreak, seawater temperatures were 1 to 5°C higher than normal in the implicated estuaries, and it was suggested that El Niño-induced weather changes may have produced higher levels (*i.e.* >11,000 CFU/g) of *V. parahaemolyticus* in shellfish.

In the following year, more than 300 *V. parahaemolyticus* infections were linked to consumption of oysters harvested from Galveston Bay, Texas (Daniels *et al.* 2000). The majority of clinical specimens yielded one PFGE profile belonging to the O3 serotype, which also possessed the virulence-associated thermostable direct hemolysin gene. Interestingly, this serotype was also associated with recent outbreaks in various geographical regions of the world. In the same year, oysters and clams harvested from the Long Island Sound in New York were implicated in 23 culture-confirmed cases of *V. parahaemolyticus* serotype O3 gastroenteritis (Wechsler *et al.* 1999). Water temperature was reportedly 8°F higher than in previous years.

The ecology of *V. cholerae* has been extensively studied in cholera-endemic regions of the world (Borrito 1998; Huq *et al.* 1990). Researchers have attempted to define environmental factors that are linked to the seasonal periodicity of cholera, and some have proposed that plankton support the growth and survival of *V. cholerae*, leading to hazardous environmental levels. Although this has not been conclusively established, new research is utilizing satellite imagery to monitor plank-

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ton blooms and define correlations with human vibrio infections (Lobitz *et al.* 2000).

CONCLUSIONS

The ecology of *Vibrio* spp. is intimately linked to estuarine and marine environments. The global impact of *Vibrio* spp. diseases is important and encourages researchers to develop effective indicators that can predict human health risks, as well as associated ecosystem integrity. Through such efforts, the interrelationships among *Vibrio* spp., human health and the environment may be better understood.

ACKNOWLEDGMENTS

This research was funded, in part, by the U.S. Department of Agriculture, U.S. Environmental Protection Agency, and the National Oceanographic & Atmospheric Administration's Sea Grant and Saltonstall-Kennedy cooperative research programs.

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