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# Proper processing destroys *V. parahaemolyticus* in seafood

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## Bacterium globally widespread in marine coastal waters



Although *Vibrio parahaemolyticus* has been found in species from scallops to lobsters, its presence is more dangerous in oysters that are consumed raw.

The first recorded outbreak of seafood infection due to *Vibrio parahaemolyticus* occurred in Japan in 1950. Of 272 patients with acute gastroenteritis, 20 fatalities occurred. The incubation period with most cases was two to six hours. The symptoms included acute abdominal pain, vomiting, and watery – in some cases bloody – diarrhea. Individuals infected with the organism suffered from other symptoms as well, such as nausea, headache, low-grade fever, and chills.

The infectious dose in the case was reported as  $10^5$  ( $1.1 \times 10^2$ ) colony-forming units (CFU). In contrast, volunteers who received much higher CFU counts ( $4 \times 10^9$ - $1.6 \times 10^{10}$ ) of an isolate of *V. parahaemolyticus* did not report any symptoms of diarrhea. Recent studies have suggested that unusually virulent strains may be infectious at lower cell numbers.

## Distribution, characteristics

*V. parahaemolyticus* is globally widespread in marine coastal waters. The bacterium is commonly found throughout brackish estuarine environments in water, sediments, suspended particles, plankton, fish, and shellfish. It is not frequently found in freshwater or full-strength seawater.

The bacterium has been isolated from 30 different marine species, including eels, crabs, clams, oysters, abalone, lobsters, scallops, sardines, shrimp, and squid. The bacterium can be isolated in higher numbers during warm summer months. It is cold-sensitive and unable to grow at the 200 to 1,000 atm hydrostatic pressures prevalent in the deep sea environment. The organism is considered to have a minimum growth temperature of 7 to 10 degrees-C and a maximum growth temperature of about 44 degrees-C.

## Detection studies

### United States

In a shellfish survey conducted by the U.S. Food and Drug Administration, 85 percent of the samples were positive for the bacterium. Concentrations of the organism were reported as high as  $1.3 \times 10^3$  CFU/gram. This survey had substantial public health significance due to the widespread practice of consuming shellfish raw or only slightly cooked.

A sampling survey of a major oyster-producing estuary in Mobile Bay in Alabama, USA, that was known to endemically harbor *V. parahaemolyticus* found the bacteria in all 156 samples collected. Pathogenic strains were detected in 22 percent of the samples. Cell densities ranged from below 10 to  $1.2 \times 10^4$  CFU/gram. During the study, higher water temperatures were associated with greater bacteria density.

### India

A survey of oysters along the southwest coast of India showed that 94 percent contained *V. parahaemolyticus* with densities ranging from less than 10 to  $6.7 \times 10^4$  CFU/gram. Pathogenic *V. parahaemolyticus* were detected in 10 percent of the isolates. Low salinity was not necessarily detrimental to the bacteria – it may instead favor growth and survival in cases where water is high in organic matter.

The India study showed that temperature was a major factor in both the seasonal and geographical distribution of the bacteria in shellfish-growing areas of the temperate region. It also showed that in tropical waters, *V. parahaemolyticus* density did not seem to be correlated with temperature. Therefore, the regression model suggested to predict the level of *V. parahaemolyticus* in the Gulf of Mexico, which is based on temperature and salinity data, does not seem applicable to tropical oysters.

### Norway

The Norwegian Food Safety Authorities conducted a two-year monitoring program covering commercially grown blue mussels. Samples were collected from July 2002 to September 2004 from 102 production sites, five of which also had wild-growing mussels, along the southern Norwegian coast. *V. parahaemolyticus* was detected in 10 percent of the blue mussels. The numbers were estimated as  $1.8 \times 10^3$  and  $2.0 \times 10^2$  CFU per gram, and the remaining samples contained less than 100 CFU per gram.

The lowest water temperature at a location where a *V. parahaemolyticus*-positive sample was collected was 0.6 degrees-C. In samples where 100 or more organisms per gram were detected, the water temperature was above 19 degrees-C. At least one isolate from each positive sample was potentially pathogenic.

## ***Italy***

In further research, mussel samples collected from approved coastal sites on the Adriatic Sea in central Italy were examined for the presence of *V. parahaemolyticus* from December 2002 to January 2004. Of the 144 samples examined, 35 were positive for the bacteria. The bacteria were isolated with a higher frequency during warmer months.

## **03:K6 serotype**

The *V. parahaemolyticus* 03:K6 serotype has enhanced virulence. It was responsible for many recent *V. parahaemolyticus* outbreaks, including epidemics in India, Russia, Southeast Asia, Japan, and North America.

Since it first emerged in Calcutta, India, in 1996, the serovar has accounted for 50 to 80 percent of the infections. In one outbreak, up to 75 percent of individuals became infected when exposed to the 03:K6 serovar, as compared to 56 percent of those infected with other serovars. In 1998, the pathogen affected 416 people in the Galveston Bay area of Texas, USA, and was the largest such incident reported in the U.S. Large epidemics of diarrhea associated with seafood consumption and *V. parahaemolyticus* occurred during the summers of 2004 and 2005 near Puerto Montt, Chile.

## **Prevention through processing**

### ***High Hydrostatic Pressure***

Several processing methods are capable of reducing levels of *V. parahaemolyticus* to non-detectable levels below 3 CFU per gram in Eastern oysters. Inactivation studies for serotype 03:K6 have been conducted in phosphate-buffered saline and inoculated oysters under high hydrostatic pressure processing (HPP) conditions.

A 6-log (99.9999 percent) reduction of *V. parahaemolyticus* in phosphate-buffered oysters at 241 megapascals (mpa) required 11 minutes, which included a three-minute pressure come-up time. A 4.5-log reduction of the bacteria in oysters at 345 mpa required 7.7 minutes, which included a 6.7 minute come-up time. The organism was reduced to nondetectable numbers at 586 mpa during an eight-minute come-up time.

Different strains of *V. parahaemolyticus* in broth cultures and inoculated Pacific oysters were also subjected to HPP treatments. Optimum conditions for reducing the bacteria to nondetectable levels in pure culture and oysters were achieved at 345 mpa for 30 and 90 seconds, respectively.

### ***Radiation, thermal pasteurization***

Gamma radiation studies using cobalt 60 in doses ranging 0.5-3.0 kGy (50,000-300,000 rads) on Brazil oysters were performed. A dose of 1.0 kGy was sufficient to produce a 6-log reduction in the level of *V. parahaemolyticus*. A dose of 3.0 kGy was effective in inactivating the bacterium without changing the oysters' odor, flavor, or appearance.

Another study used thermal pasteurization to reduce counts of *V. parahaemolyticus* 03:K6 to nondetectable levels. Oysters were artificially contaminated with 104-106 CFU/g of the 03:K6 serovar, which proved more process-resistant than nonpathogenic strains found in Gulf of Mexico waters. A total processing time of at least 22 minutes at 52 degrees-C was recommended to reduce the bacteria to levels less than 3 CFU/gram.

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