Vibrio cholerae and its Significance in Seafoods

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Cholerae has been endemic in Eastern India and in East Pakistan (present Bangladesh) since the beginning of recorded history. The fact that this disease is caused by an organism, *Vibrio cholerae*, was first reported by Robert Koch in 1884, who observed that a wide variety of *Vibrios* commonly found in nature were characteristically haemolytic, whereas true *Vibrio cholerae* were not haemolytic. But subsequently, in 1906, haemolytic strains of *V. cholerae* were isolated from dead bodies of pilgrims seen at the El Tor quarantine station in Egypt. The cholera outbreak in Sulawesi (Indonesia) in 1939 was found to be due to these haemolytic biotypes (El Tor biotype) of *Vibrio cholerae.* The marked epidemiological difference between El Tor and classical cholera are that the infection-to-case ratio is higher with El Tor cholera and the El Tor *Vibrio* is generally surviving longer in the environment making it more easily detectable.

Morphology

Vibrio cholerae are shaped like a coma or a curved rod measuring 1 to 5 micron in length and 0.3 to 0.6 micron in breadth. They are gram negative, non-spore forming and are actively motile by a single polar flagellum. It is aerobic and the optimum growth temperature is 37°C. It is one of the most rapidly multiplying bacteria outgrowing other organisms in the early hours of incubation. It is unusually tolerant of alkali, growing in media as alkaline as pH 9.2, a property utilized for primary isolation of this organism. On prolonged cultivation, *Vibrios* may become straight rods resembling other gram negative enteric bacteria.

Natural habitat

The only known natural reservoir of *V. cholerae* is man and it is transmitted from man to man through the environment. Usually food,

water, flies and contaminated hands play prominent role in the transmission of this organism. The infected individual will usually secrete *Vibrios* for only a few days. But a few chronic carriers (persons harbouring the organism for more than 3 months) have been reported, and one individual has been reported to have been infected for 7 years.

Pathogenesis of *V. cholerae*

In the cholera victim, the organisms gain entry through mouth into the small intestine where the alkaline medium and an abundance of products of protein metabolism furnish favourable conditions for their multiplication. The organism on multiplication in the small bowel produces an exotoxin which acts upon the mucosal cells of the small bowel causing them to secrete large quantities of isotonic fluid. The small bowel produces isotonic fluid faster than the colon can absorb it resulting in a watery isotonic diarrhoea. This rapid gastro-intestinal loss of isotonic fluid is responsible for all the clinical manifestations of the disease.

Clinical symptoms of disease

After an incubation period of 1-4 days, there is a sudden onset of nausea and vomiting and profuse diarrhoea with abdominal cramps. The stools resemble "rice water" and contain mucus, epithelial cells and large numbers of *Vibrios*. There is rapid loss of fluids and electrolytes, which leads to profound dehydration, circulatory collapse and anuria. The mortality rate without treatment is between 25 and 50%.

Different serotypes of *V. cholerae*

The *V. cholerae* is set apart serologically from other *Vibrios* by its specific O (heat stable) antigen. Three major O antigens can be differentiated between O - group 1 and have been designated A, B and C. Of these, the antigen 'A' is considered to be O - group 1 specific antigen. It occurs in combination with the other antigens to give *Vibrio* serotypes. The antigen combination AB is the Ogawa serotype, the combination AC the Inaba serotype and the combination ABC the rare Hikojima serotype.

NAG *Vibrios*

NAG (non-agglutinable) *Vibrios* possess biochemical and morphological characteristics very similar to those of the cholera *Vibrio,* but are non-agglutinable with polyvalent 'O' serum of the cholera *Vibrio.* Such *Vibrios* are agglutinable by their own antisera and may produce cholera like or mild diarrhoea.

Seafood - related outbreaks of cholera

An explosive outbreak of *V. cholerae* El Tor has been reported in Philippines during 1961 and 1962. The initial infection was mainly from shrimps that were consumed raw. In 1969, another outbreak occurred in Malaysia. El Tor and non-agglutinating (NAG) strains were isolated from water and shellfish. A cholera epidemic caused by El Tor biotype began in Naples in 1973 and resulted in 25 deaths among 278 bacteriologically confirmed cases. The suspected vehicle of transmission was raw seafoods, particularly mussels, washed with dockside seawater contaminated with *V. cholerae*. One year later, 48 deaths occurred in Portugal on consumption of shell fishes and the causative organism was *V. cholerae* biotype El Tor. Forty two per cent of the shell fish samples were found to be infected with *V. cholerae*. During the same period, six cases of cholera occurred in Guam and were associated with homepreserved fish. In the United States, the first case of cholera since 1911 was reported in Texas in 1973. Although the source of infection was undetermined, the individuals had consumed raw oysters. In 1977, a similar case occurred in Alabama in an individual who had eaten large quantities of raw oysters. A cholera outbreak transmitted through boiled crab meat has been reported in Lousiana in 1978. In a Food and Drug Administration (FDA) investigation, samples of blue crab from suspected areas revealed numerous non-agglutinable strains of *V. cholerae*.

Thus, it is clear that mostly shellfishes and crustaceans are involved in cholerae outbreaks but a finfish was incriminated in the Guam outbreak. In the eastern hemisphere, cholera usually infects impoverished people who live in unsanitary conditions. However, in the West, cholera often occurs

among the middle class and is usually more severe in individuals with gastric disorders.

Survival of *V. cholerae* in seafoods and environments

The survival of *V. cholerae* under various environmental conditions has been investigated by many workers. As early as 1959, the survival of *V. cholerae* in seawater has been demonstrated. In general, survival was enhanced by intermediate salinities, lower temperature, high organic content, neutral pH, dark storage and absence of competing microflora in the substrate. Survival time of the organism has been reported to be 47 days in unsterilized bay water compared to 7 days for ocean water. But, survival for more than 285 days has been observed both in ocean and bay water sterilized before being inoculated with *V. cholerae.*

Survival of *V. cholerae* in seafoods has also been investigated. Japanese workers have demonstrated uptake of *V. cholerae* by oysters and clams kept in cholera-polluted seawater. The *Vibrios* entered the gastro-intestinal tract of the shellfish and survived for 1.5 months at 0 to 5°C and 15-20 days at 22°C. In oysters smeared with *V. cholerae* and stored at 20°C, the number of organisms first decreased, then increased with the maximum number occurring after 68 hours. A gradual decrease followed and most of the *Vibrios* disappeared by 171 hours. The survival period of the *Vibrio* increased to about 20 days in oysters and clams that had been sterilized or boiled before contamination. *V. cholerae* survived only for a few days in fish stored at room temperature but persisted for more than 3 weeks when refrigerated. Survival of the *Vibrio* for 198 days in "sea salt solution" has been reported; again, at lower temperature there is extended survival. El Tor *Vibrios* survived for more than a month in various foodstuffs frozen at -20°C and much longer in foods frozen at -72°C.

There are also reports that, in summer months *V. cholerae* are also associated with zooplankton and that chitinase activity in *Vibrios* plays a role in this association. The chitinase activity may increase the affinity to crustacea, thus explaining why *V. cholerae,* a chitin digester, is found more frequently in crabs and shrimps than in other seafoods.

It is thus clear that the seawater and seafoods are sometimes contaminated with *V. cholerae* and that the organisms can survive in these substrates for a pretty long time.

Control measures

It is clear that environmental hygiene and sanitation play a significant role in the contamination of seafoods with *V. cholerae*. Therefore considerable stress has to be given for environmental sanitation and hygiene. The surroundings of the processing plant should be kept clean and disinfected. Only potable water supply should be used and the water used for different purposes should be properly chlorinated. As flies, cockroaches etc. have been implicated in the transmission of various alimentary infections including cholera, measures should be taken to combat these insects in the processing premises. Further, more stress should be given to personal hygiene of the fish handlers by providing sufficient number of urinals and by providing hand and feet washing and disinfecting facilities. It is also advised to have periodical medical check-ups of the workers engaged in processing and other operations.