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Human enteric viruses in shellfish, part 1

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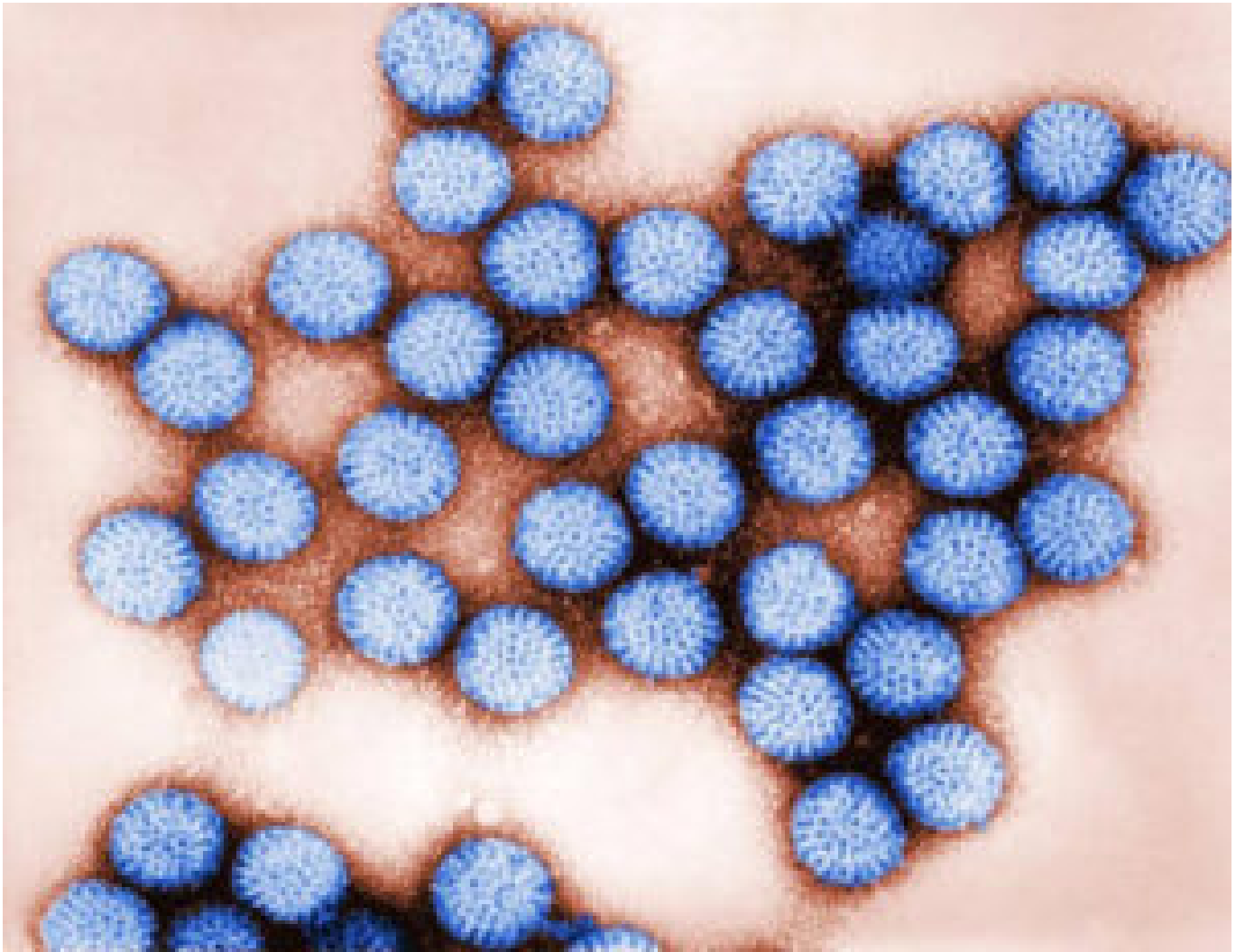
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Rotaviruses

Viruses pathogenic to humans have been detected in shellfish harvested from approved waters. Based on the fecal coliform indicator and the presence of *Escherichia coli*, commercially harvested shellfish, oysters, mussels, clams and cockles have been associated with gastroenteritis outbreaks.

Contamination occurs from discharges of treated and untreated sewage effluents, unintentional discharges by urban runoff and waste inputs from boats, and via rivers when wastewater discharges occur in freshwater. Also, contamination from wild and domestic animal sources have been significant contributors, as well.

Until recently, it was difficult to assess viral contamination and its potential impacts on public health. The main pathogenic enteric viruses able to survive and persist in the environment and contribute to outbreaks are the enterovirus, hepatitis A virus, Norwalk-like virus, rotavirus and astrovirus.



Infectious rotaviruses can be found in many shellfish samples and growing waters worldwide. Human population density greatly influences the contamination levels.

Detection

Technological advances in molecular detection methods have led to the development of sensitive, specific assays, such as reverse transcription polymerase chain reaction (RT-PCR) and hybridization, for the detection of viruses, including those that grow poorly or not at all in cell culture, including Norwalk-like virus, hepatitis A virus and rotavirus. Moreover, preliminary steps, such as concentration of viruses from samples and nucleic acid purification, are essential for final PCR accuracy and reproducibility.

Different methods have been proposed for determining viral contamination based on whole shellfish or dissected tissues for all types of virus. The method based on dissected tissues is considered to be specific, reliable and reproducible, providing a nucleic acid extract that allows detection of most enteric viruses.

Rotavirus disease is most common in infants and young children. However, older children and adults can also become infected with rotavirus from consumption of contaminated shellfish. Once a person has been exposed to rotavirus, it takes about two days for the symptoms to appear. Vomiting and watery diarrhea can last from three to eight days. Additional symptoms include loss of appetite and dehydration, which can be especially harmful for young people, the elderly and immunocompromised. Healthy adults who contract rotavirus disease tend to have milder symptoms when compared to others.

European studies

Various studies have been performed to examine viral contamination. In a study in France, oysters, *Crassostrea gigas*, and mussels, *Mytilus galloprovincialis*, were collected from three sites subjected to occasional bacterial contamination confirmed by fecal coliform counts. Bacterial contamination above the European standard of 300 fecal count/100 g was detected in nine samples, and *Salmonella* was detected in one sample.

Viral contamination was found mainly during the winter season. Nine of the 11 oyster samples were contaminated by more than three types of viruses. Rotavirus was detected in 30 samples, representing 27 percent of the tested animals.

In areas subject to sewage discharge, mussels were prohibited for collection and human consumption. At one sampling site, 13 samples were negative, and 23 were positive for viral contamination. Sixteen of the samples contained rotaviruses. Single-virus contamination was detected in two rotavirus samples.

At another site, 13 of 37 samples were contaminated by at least three types of viruses. Rotavirus was detected alone in three samples and with other viruses in 22 samples. Viral detection in both the oyster and mussel samples was seen mainly during the cold months of November through March.

A study was conducted on human enteric virus occurrence in shellfish in European markets. In Spain, the study was conducted on both the Atlantic coast and the Mediterranean Sea. As anticipated, within each of the 11 sampling areas on the Atlantic coast, the human population density showed a clear influence on the contamination levels of the samples.

Rotavirus was detected in a low percentage of the samples. No rotavirus was reported from samples obtained from the Mediterranean Sea. Samples taken from Italian waters contained a rotavirus contamination of 32 percent.

In Greece, no rotavirus was detected in shellfish obtained from various growing areas, and with or without depuration. However, 100 percent of retail samples tested positive for rotaviruses.

Shellfish samples analyzed for rotavirus contamination from the United Kingdom, Ireland, the Netherlands, Norway, Sweden, Denmark and Germany were negative.

North, South American studies

Samples of water and *Crassostrea gigas* oysters were taken from the southern region of Brazil to provide viral contamination data for further epidemiological studies and governmental actions. In 84 water samples taken from June through May of the following year, 19.0 percent were positive for rotavirus. Infectious rotavirus contamination was confirmed at 12.5 percent.

The objective of another study in Brazil was to evaluate water samples and mussels, *Mytella guyanensis*, collected in a mangrove area characterized by a continuous discharge of domestic sewage. Although *Escherichia coli* was detected at low densities in water samples, mussels were shown to be more than 400 times more contaminated throughout the study period. Rotaviruses were detected in 88 percent of the water samples and 100 percent of the mussel samples. The high rates of contamination were anticipated.

In Mexico, samples of *C. virginica* oysters were collected in Mexico City to determine the presence of rotavirus genotypes and evaluate their potential to produce gastrointestinal infections. Out of 63 samples, 16 percent of the oysters tested positive for rotavirus contamination.

Asian studies

A viral pollution study was conducted in China in 45 primarily commercial shellfish-growing areas near coastal cities. A total of 162 samples were collected during the month of August. The samples consisted of 91 clams of seven species, 19 *Sinonovacula constricta* and *Solen grandis* razor clams, 18 *Mytilus edulis* and *Perna viridis* mussels, 17 *Crassostrea gigas* and *C. plicatula* oysters, nine blood clams of three species and eight *Pecten farreri* and *P. yessoensis* scallops.

Using polymerase chain reaction analysis, rotavirus was detected in 7 percent of the samples: eight clams, two razor clams and one blood clam. No rotavirus was detected in oyster, mussel and scallop samples.

In other research, the prevalence and molecular characterization of rotaviruses in Thailand were examined. A total of 114 water samples and 110 *C. belcheri* oyster samples were collected and tested for group A rotavirus.

The rotavirus was detected in 21 water samples and six oyster samples. Twenty-five rotavirus strains were classified into four genotypes: G1, G2, G3 and G9. G1, G2 and G9 strains demonstrated genetic sequences similar to human strains, whereas G3 was closely related to animal strains.

In South Korea, viruses were collected from 152 shellfish samples – 51 *C. gigas* oysters; 51 Manila clams, *Tapes philippinarum*; and 50 *Mytilus coruscus* mussels. While other viruses were present, including norovirus, hepatitis A virus and hepatitis E virus, no rotavirus was detected.

Perspectives

As reflected in the results of the above studies, rotaviruses are anticipated to be present in many shellfish and their growing waters. Infants and young children are most affected by rotavirus-caused gastroenteritis, although they are not customary consumers of shellfish.

Adults may suffer illness from rotaviruses, but since their symptoms are not usually severe, the illnesses are often not reported to health authorities. However, as our population continues to age and more individuals are immunocompromised, regulatory agencies having jurisdiction in shellfish safety may conduct additional research to determine the health risks rotaviruses present and propose regulations accordingly.

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