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Human enteric viruses in shellfish, part 4

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An examination of norovirus prevalence in Europe

As reported in previous articles in this series, noroviruses are the major cause of outbreaks of acute gastroenteritis in humans. Consumption of shellfish is one of three main transmission routes of norovirus infection.

This article will discuss the viruses' presence and significance in shellfish produced in various countries in Europe. It is important to note that some molluscan shellfish in the study reporting were obtained from prohibited areas, areas known as receiving untreated sewage or from growing areas classified as B and C by the European Union Commission. Therefore, some of the studies may not reflect the potential for human illnesses due to harvest restrictions for direct human consumption.



In studies, shellfish raised under different cultivation methods in the same affected area were similarly involved in virus contamination.

Italy, various species

A study in Italy carried out from 2003 to 2011 was initiated to define the prevalence of norovirus contamination in 4,463 samples collected from commercial shellfish production areas designated for direct human consumption. Samples included 2,310 mussels (*Mytilus galloprovincialis*, *M. edulis*); 1,517 clams (*Tapes* species, *Callista chione*); 510 oysters (*Crassostrea gigas*, *Ostrea edulis*); 22 minor species (*Ensis* and *Murex* species, *Pecten jacobaeus*, *Tellina* species, *Agropecten purpuratus*, *Chamelea gallina*, *Glycineris* species and *Venus verrucosa*) and 104 samples of preserved fish salads, fish fillets, fresh or frozen fish, squid, shrimp and prawns.

Among the samples analyzed, 86.7 percent were domestic, and 13.3 percent were imported. About half of the samples were collected in the Veneto region on the Adriatic Sea, while the remainder was collected in Emilia-Romagna, Liguria and other major coastal regions: Marche, Puglia, Campania, Sardinia and Sicily.

The average positivity rate for norovirus presence was 4.1 percent and ranged from 0.6 percent in 2007 to 9.8 percent in 2003. The values ranged from 1.9% in preserved products to 4.7 percent in mussels. Genetic characterization showed a prevalence of genogroup II genotypes, including GII.b, GII.e and different GII.4 variants.

Switzerland, oysters

To assess the percentage of virus-contaminated *Crassostrea gigas* and *Ostrea edulis* oysters imported into Switzerland, 87 samples consisting of five oysters each were analyzed for the presence of noroviruses from November 2001 to February 2002.

Sixty-one of the samples were exported by 31 different French suppliers, 12 of the samples were exported by three Dutch suppliers, and 14 of the samples were exported by two Irish suppliers. Eight of the 87 samples from six of the 31 French suppliers were positive for noroviruses.

France, oysters

A total of 387 oyster samples harvested in France between February 2010 and May 2011 were collected from 42 different locations based on market availability. Among the samples, 149 were produced on the southwestern coast, and 72 came from the western coast at Brittany-Normandy. The production locale for the remaining 167 samples (in supermarkets) could not be identified due to information contained on the sanitation label. Among the 387 samples, 91 percent were norovirus-negative, and 9 percent were contaminated with norovirus (Table 1).

Sampling site	Collected	Negative samples	Positive samples
All	387	352	35
Supermarket	82	68	14
Packers	141	127	14

Sampling site	Collected	Negative samples	Positive samples
Producers	164	157	7

One hundred five samples were produced in higher-quality areas with less than 230 *Escherichia coli*/100 g of shellfish tissue. Three were put on the market within two days, and the other 102 samples were relocated to basins adjacent to the farms with seawater changed twice a day via tidal events. The other 282 samples were produced in class B areas and underwent depuration prior to analysis.

Ireland, oysters

In a study undertaken in Ireland to investigate norovirus contamination in oysters (*Crassostrea gigas*) from a shellfishery over the 24-month period from October 2007 to September 2009, 18 oysters were obtained monthly from an area closed due to a previous norovirus outbreak. The harvest area may have been impacted by discharges from wastewater treatment plants, including one located approximately 1 km from the harvest area that provided ultraviolet disinfection in addition to secondary treatment, and another about 10 km from the site that provided secondary treatment only.

Total GI and GII norovirus concentrations in norovirus-positive oysters ranged 97-20,080 genome copies/g of digestive tissue and displayed a strong seasonal trend, with greater concentrations during the winter months. The norovirus concentrations were similar during both years of the study.

Although norovirus GII.4 is responsible for the vast majority of outbreak reports, multiple norovirus genotypes were identified during the study: GI.4, GI.3, GII.4, GII.b, GII.2 and GII.e. Norovirus GI.4, the most frequently detected genotype, was present in 99.9 percent of positive samples. This was followed by GII.4 at 43.7 percent and GII.b at 37.5 percent of positive samples.

The data demonstrated the diversity of norovirus genotypes that can be present in sewage-contaminated shellfish. A disproportionate number of non-norovirus GII.4 genotypes can be found in environmental samples compared to the number of recorded human infections associated with non-norovirus GII.4 genotypes.

Portugal, various species

Approximately 2,000 bivalve shellfish consisting of Asian clams, *Curbicula fluminea*; native clams, *Ruhitapes decussates*; tellina clams, *Tellina crassa*; surf clams, *Spisula solida*; clock clams, *Dosinia exoleta*; razor clams, *Ensis* species; mussels, *Mytilus* species; flat oysters, *Ostrea edulis*; and cockles, *Cerastoderma edule*, were collected from 10 harvesting areas classified from A to C in the north and center of Portugal from March 2008 and February 2009. The shellfish were grouped into 49 batches based on species and collection sites.

Viral contamination was detected throughout the year in all shellfish species and in all collected areas, independently of their harvesting area classifications. Overall, 67 percent of all analyzed batches were contaminated by at least one of the studied viruses – norovirus, hepatitis A virus and enterovirus – while the simultaneous presence of two and three viruses was detected in 22 percent and 6 percent of the batches, respectively. Of the three viruses, norovirus was detected in 37 percent of the batches. All strains belonged to genotype GII.4.

Spain, mussels

A total of 81 mussel samples were obtained over the 18-month period from October 2010 to March 2012 from seven harvesting areas in Rio do Burgo, A Coruna in northwestern Spain. Five of the harvesting areas were classified as B, and two were classified as C.

Noroviruses were detected in 49.4 percent of the cases, reaching contamination levels from 5.9×10^3 to 1.6×10^9 copies/g digestive tissue for GI and from 6.1×10^3 to 5.4×10^6 copies/g digestive tissue for GII. Norovirus strains were assigned to genotypes GI.4, GII.4 and GII.6.

Turkey, mussels

Mussels were collected along the Bosphorus Coast near Istanbul, Turkey, during 2008-2009. Of the 320 mussel samples collected, five were found to be positive for norovirus genotype GII. No genotype GI was detected. Norovirus genotype GII was present in samples collected in October, November and December 2008, and February and July 2009.

Poland, mussels

Wild mussel samples were collected from three sites along the Polish coast in the Baltic Sea. In total, 120 shellfish were tested as pooled samples. Norovirus GI was detected in 22 (18.3%) of the samples, and norovirus GII was detected in 28 (23.3 percent) of the shellfish. Nucleotide sequence analysis of the detected norovirus GII strains showed 97.3 percent to 99.3 percent similarity to the GII.4 virus strain.

Belgium, various species

Shellfish and fishery products in Belgium were analyzed from October 2012 to March 2013 for the presence of norovirus. For the intact oysters, mussels and clams, 21 of 65 samples from 12 of 34 batches were positive for noroviruses. Nine samples contained quantitative norovirus levels at 3,300-14,300 copies/g shellfish tissue.

For semi-processed scallops and common sole rolls with scallop fragments, 29 of 36 samples from all eight batches were positive for noroviruses. Seventeen samples contained quantitative noroviruses levels at 200-1,800 copies/g tissue. Norovirus genotypes GI and GII were identified in samples, while some samples contained both GI and GII.

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