

Norovirus in bivalve molluscs: a study of the efficacy of the depuration system

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Summary

Noroviruses are the most common viral agents of acute gastroenteritis in humans and are often associated with the consumption of either fresh or undercooked live bivalve molluscs. The aim of the study was to evaluate the efficacy of the water depuration systems in the presence of Norovirus contamination. A total of 96 shellfish samples was examined by reverse transcriptase-polymerase chain reaction, as follows: 58 mussel samples (*Mytilus galloprovincialis*), 35 Manila clam samples (*Tapes decussatus*) and 3 Pacific oyster samples (*Crassostrea gigas*). Of these, 67 were collected before and 29 following depuration. Viral RNA was detected in one of the 67 non-depurated samples examined (1.5%; 95% confidence interval: 0.36-7.92%) and in one of the 29 depurated samples (3.4%; 95% confidence interval: 0.82-17.22%). There were no statistically significant differences between depurated and non-depurated samples which indicated that the purifying systems in place were not able to remove Norovirus contamination from the live bivalve molluscs.

Keywords

Bivalve mollusc, Depuration, Italy, Mollusc, Norovirus, Norwalk, Public health, Virus.

Introduction

The Noroviruses, previously known as Norwalk-like viruses (NLV), are a group of non-enveloped viruses with a positive RNA strand of about 7.6 kb containing 3 open reading frames (ORFs). They belong to the family *Caliciviridae*, genus *Norovirus*, and are currently classified in five genogroups (GI, GII, GIII, GIV and GV), of which GI, GII and GIV are capable of infecting humans, and 31 genetic clusters (4). They are becoming increasingly important for public health as a cause of non-bacterial acute gastroenteritis in school-age children and adults. They are most frequently spread from person to person, but contaminated food and water can also play an important role (8). Infection is often associated with the consumption of bivalve molluscs which, due to their filtering capacity (a mussel can filter up to 1.5 l of water per hour at 14°C), accumulate and concentrate different types of pathogens in their tissues, especially in the stomach and digestive glands (1, 14); the virus is also able to resist normal depuration treatments that molluscs undergo and, under optimal conditions, can survive for a long time in the environment (7).

This study aimed to detect Norovirus in bivalve molluscs destined for human consumption and to evaluate the effect of current depuration systems.

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Materials and methods

Sampling

This study focused mainly on an important depuration plant in Termoli which handles most of the molluscs harvested along the Abruzzi and Molise coastline. Molluscs were sampled on the basis of the volume of processed products. Both depurated and non-depurated samples were collected to evaluate the safety of the products destined for human consumption and the effects of depuration treatment on Noroviruses.

Each sample comprised 5 kg of mussels. For each sample, the type and place and date of collection were recorded. To have a more comprehensive view on the safety of the products of the entire study area, batches from the depuration centre of Giulianova, which processes the products from the coastal area of the Teramo Province, were also collected and tested.

Ninety six mollusc samples were collected and tested as follows:

- 58 mussels (*Mytilus galloprovincialis*) of which 47 were non-depurated and 11 were depurated
- 35 Manila clams (*Tapes decussatus*), of which 20 were non-depurated and 15 were depurated
- 3 depurated oysters (*Crassostrea gigas*).

The depuration plants involved in the study utilised either traditional or closed-circuit depuration systems. In both, however, the water was disinfected using UV light rays.

Virological tests

For each sample 3 aliquots of 1.5 g of the stomach and digestive glands were tested. All samples were tested for the presence of the Norovirus using reverse transcriptase-polymerase chain reaction (RT-PCR) with the proteinase K and hexadecyltrimethylammonium bromide (CTAB) extraction procedure and a commercial kit (Access RT-PCR System, Promega), with primers JV12 and JV13.

An internal control of the reaction (Armored RNA[®] Norwalk Virus Asuragen[®] Inc., Austin,

Texas), was added to the sample to be tested to check for any RT-PCR inhibition phenomena. The sample was then amplified with a suitable primer set (NLV GI SR33: 5'-TGT CAC GAT CTC ATC ATC ACC-3', SR48:5'-GTG AAC AGC ATA AAT CAC TGG-3') using an RT-PCR reaction with the following thermal profile: 48°C for 60', 94°C for 5', 40 cycles of 1' at 94°C, 1'30" at 50°C, 1' at 74°C; 74°C for 7'. The size of the amplification product was found to be 123 bp by fluorescent staining with ethidium bromide.

Statistical analysis

The virological data were analysed by using the beta distribution ($s+1$, $n-s+1$) where s , the number of successes, is the total number of positives and n , the number of tests, is the total number of samples examined. The peak of the distribution curve represents the most likely value for the percentage of positive samples, while its width indicates the uncertainty of the estimation, due to the sample size.

Results

A similar percentage of positive samples was found for both mussels (1.7%, 95% confidence interval [CI]: 0.41-9.09%) and clams (2.9%, 95% CI: 0.68-14.53%) (Fig. 1). The three oyster samples were negative. No significant differences were observed between depurated and non-depurated samples (Fig. 2). Norovirus RNA was found in one of 67 non-depurated samples (1.5%, 95% CI: 0.36-7.92%) and in one of the 29 samples examined after depuration (3.4%, 95% CI: 0.82-17.22%). The two positive samples included one non-depurated Manila clam sample (5%, 95% CI: 2.1-38.5%) and one depurated mussel (9.1%, 95% CI: 2.1-38.5%). Figure 3 provides the probability distributions for detection of a Norovirus positive sample in depurated and non-depurated mussels and clams.

Discussion and conclusions

The presence of Norovirus was revealed by the identification of viral RNA in clams and mussels farmed or harvested along the Abruzzi Adriatic coastline. This demonstrates

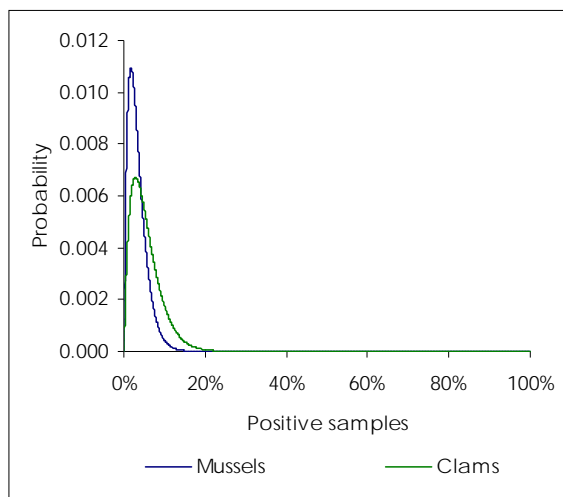


Figure 1
Probability distribution of the percentages of mussels and clams positive to Norovirus reverse transcriptase-polymerase chain reaction

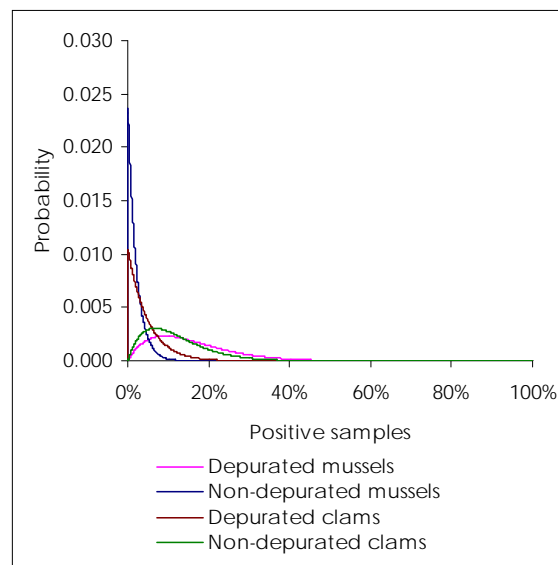


Figure 3
Probability distribution of the percentages of mussels and clams positive to Norovirus reverse transcriptase-polymerase chain reaction before and after depuration

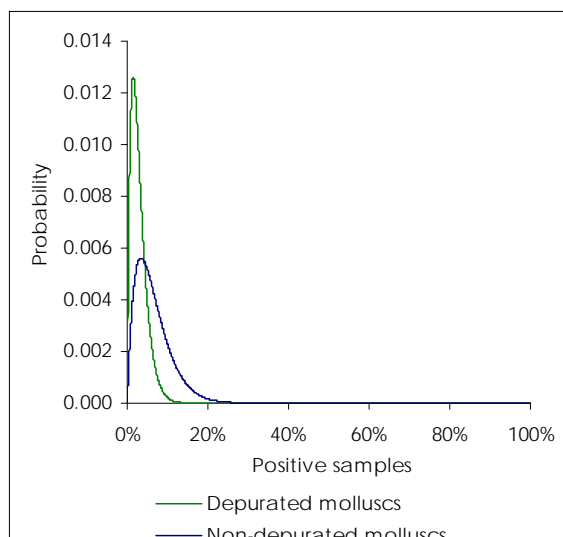


Figure 2
Probability distribution of the percentages of molluscs positive to Norovirus reverse transcriptase-polymerase chain reaction before and after depuration

that Norovirus represents a potential risk for human viral gastroenteritis in the Abruzzi. An outbreak of gastroenteritis affecting a large number of people on the Abruzzi coast was recorded between July and September 2003; faecal samples processed using the enzyme-linked immunosorbent assay (ELISA) and RT-PCR revealed the presence of Norovirus genogroups I and II (17). Several other outbreaks were reported in southern Italy (2, 13, 15).

The most common source of Norovirus infection in humans is through the consumption of foods that have come into contact with water contaminated with human faecal matter from sewer discharges. The virus spreads through surface, marine and sedimentary waters (6) and hence to foods coming into contact with them, such as bivalve molluscs (5). This study also demonstrated that the current depuration systems, such as those used in the Termoli plant, have no effect on the purification of contaminated mussels from Norovirus. As reported by other authors, no significant differences were found between the presence of Norovirus in depurated and non-depurated mussels in this study (18, 19). Ueki *et al.* found that the mean concentration of Norovirus in contaminated oysters was not significantly lower 3 and 10 days after depuration compared to that found in oysters farmed in non-depurated waters (19). This scenario is also reported for some virus types, including hepatitis A (9), while for others, such as the enterovirus, water depuration does significantly reduce the viral load in contaminated molluscs (12). The persistence of Norovirus in oysters after depuration could be attributed to specific virion binding with the surface carbohydrates expressed by the oyster

tissues, as described by Le Guyader *et al.* (10). There is thus a real possibility that molluscs contaminated with Norovirus might reach the consumer.

Cases of Norovirus due to contaminated food consumption are rising significantly. Similarly, losses due to the presence of this virus are reported increasingly in mussel farms (19). Molluscs become contaminated through their normal filtration process, and the virus can remain in them for several weeks (10). In oysters, the presence of virus may also depend on other environmental factors, such as water temperature, the mucus of the oyster and the glycogen content of connective tissue (3).

Given the widespread distribution of these viruses and the consequent importance of these infections for public health because of their high infectivity, their repercussions on dietary habits, such as the consumption of raw or relatively slightly cooked molluscs, the risk posed by an infected person to other people

and, last but not least, the financial losses caused by the epidemics, the implementation of control measures is advisable, even if the health risk is low.

Although it is currently impossible to remove or inactivate Norovirus in bivalve molluscs through water depuration systems, the use of further preventive measures, such as monitoring production areas to evaluate the actual prevalence of the virus, identification of at-risk areas and the development of safer depuration systems, as well as preventive treatment of raw sewage, are all essential. It would also be preferable to farm molluscs in coastal areas that are not affected by domestic water discharge which is the greatest source of contamination (11, 16).

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