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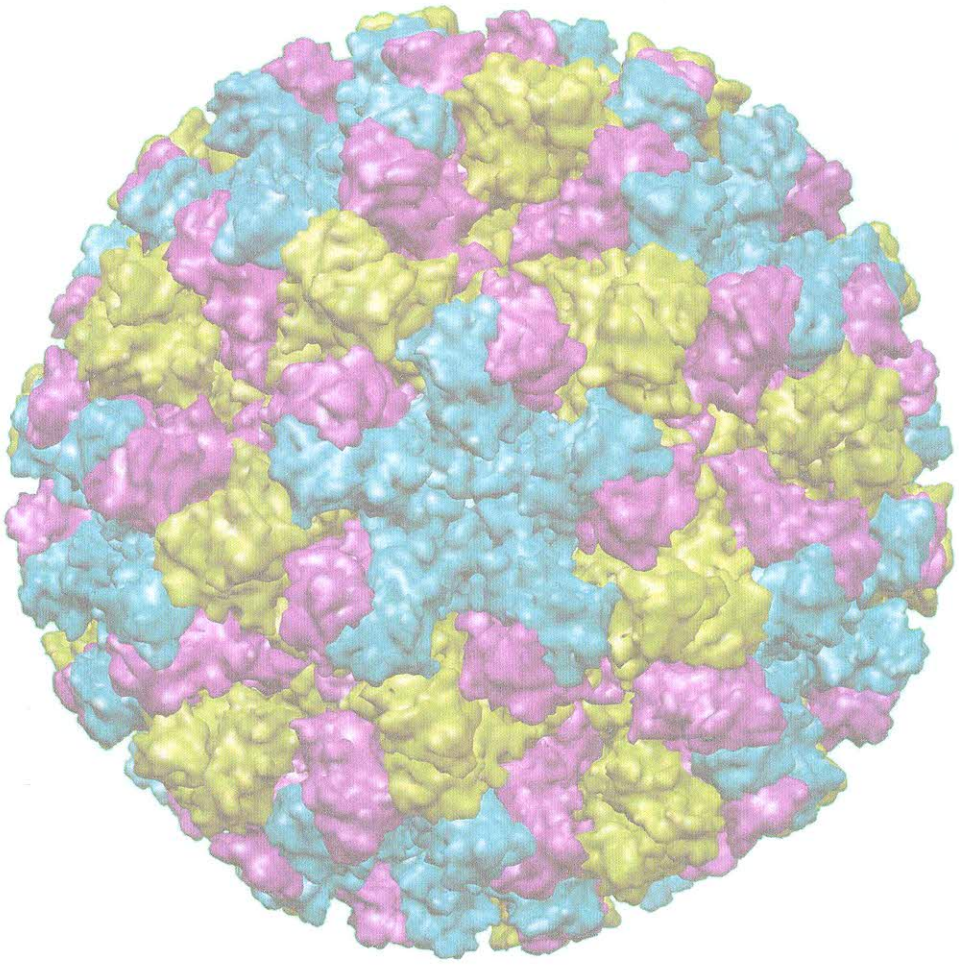
Norovirus and Other Caliciviruses on the Rise
International Conference



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P15 – Norovirus GI and GII Prevalence in Bivalve Molluscs and Vegetables in Italy

Enrico Pavoni[1], Simona Di Pasquale[2], Dario De Medici[2], Marina Nadia Losio[1], Elisabetta Suffredini[2], Barbara Bertasi[1] & Luciana Croci[2]

[1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emila Romagna, Brescia, Italy
[2]Istituto Superiore di Sanità, Rome, Italy

In recent years, Noroviruses (NoV) are considered the major cause of non bacterial gastroenteritis in industrialized countries, although there is still reason to believe that their number is underestimated. According to the nucleotide sequence of the NoV capsid region, they are divided into 5 genogroups (GI, GII, GIII, GIV and GV), which in turn contain several clusters or genotypes. The majority of strains infecting humans belongs to genogroup I, with 14 genotypes, and the genogroup II, with 17 genotypes (1). Transmission may occur by "person to person" contact or by consumption of contaminated water or food. In this context, bivalve molluscs and raw or ready to eat (RTE) vegetables play a special role. It is important to note that Italy is the third European producer of molluscs, after Spain and France and every year 180,000 tons of molluscs are harvested in Italy (2). Despite the epidemiological evidences, the current European legislation (EC Reg. 1441/2007) included no microbiological criteria for the control of viral contamination in foodstuffs. The availability of molecular methods based both on conventional PCR (i.e. RT-booster-PCR), or on Real-time PCR, permitted to assess the prevalence of NoV GI and GII in these types of foods.

We report data obtained from surveys carried out on bivalve molluscs collected on import sites and on markets. Moreover, two surveys were carried on to verify NoV contamination in vegetables. Of these, the first was conducted in Lombardia region (Northern Italy) on samples from the market, while the second was done in Lazio region (Central Italy) on samples from two companies, differing for their microbial stabilization technology (cryogenic removal and disinfection with halogens respectively). The total number of analysed bivalve mollusc samples was 872. The 6.4% of samples collected on markets was positive, while 29.3% of samples collected on import sites were contaminated by NoV. Vegetables samples from Lombardia region were 297, and 6 samples (2.02%) were positive for NoV: in three samples was confirmed the presence of NoV GII/4. In the monitoring carried out in Lazio region 124 samples were analysed (46 raw materials and 78 packaged RTE products analysed on their last day of shelf life). All samples were negative for Norovirus.

These data underline the need to integrate the European Community legislation on microbiological criteria for possible virus contamination in foodstuffs. Furthermore, it is important that the food business operators, both in case of import or in case of primary production, take into account the possibility of a viral contamination, and improve their HACCP programs in order to ensure that food safety is not compromised.

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P16 – Full Year Norovirus Surveillance Study on Source Water for Four Drinking Water Treatment Plants in Sweden

Fredrik Nyström[1,2], Olaf Dienus[2], Johanna Ansker[3] & Per-Eric Lindgren[1,2]

[1]Dept. of Clinical and Experimental Medicine, Medical Microbiology, Linköping University, Linköping, Sweden, [2]Medical Diagnostics, Microbiological Laboratory, Ryhov County Hospital, Jönköping, Sweden, [3]Stockholm Vatten AB, Stockholm, Sweden

Norovirus (NoV) has been indicated as the most important viral pathogen in future waterborne disease outbreaks both by the WHO in *Guidelines for Drinking-water Quality*[1] and in the Swedish Government Official Report, *Climate and vulnerability SOU 2007:60.*[2]
NoV has been the cause of several waterborne disease outbreaks in Sweden[3], and still little is known about background levels of NoV in source water. Background levels of pathogens are required to perform a reliable risk assessment, a valuable tool to improve the water treatment. Traditionally Swedish drinking water treatment plants (DWTP) were modeled to remove mainly fecal bacteria, but a greater awareness regarding protozoal and viral contamination has increased the demand for additional treatment steps.
In this study source water for four large municipal DWTPs in Sweden was sampled every two weeks for one year (September 2010 – October 2011). The sample sites included are two locations in Lake Mälaren, the third largest lake in Sweden with a very low water flow. Göta älv, a large river in western Sweden with a high flow, and Lake Ringsjön in southern Sweden with a low water flow. In total the four DWTPs provide clean drinking water to over 2,500,000 consumers.
Each sample was subjected to a three-step isolation method of virus particles. Firstly, a two-step filtration procedure using neutral and charged filter membranes was applied. Secondly, ultrafiltration using a centrifugal microconcentrator was conducted. Murine norovirus (MNV-1) was used as a process control for entire isolation procedure. Viral nucleic acid extraction was performed on the remaining retentate, followed by cDNA-synthesis using random hexamer primers. A TaqMan real-time PCR assay was used to detect and quantify concentration of NoV genogroup one (GI) and genogroup two (GII).
NoV GII was detected from early October 2010 to early April 2011 with the highest quantifiable titer in early January 2011. Scattered findings of NoV GI were detected during the same time frame. The concentrations of NoV from this study will be used as input values in a Quantitative Microbial Risk Assessment (QMRA) model to improve the quality of the simulations and thus obtaining a better understanding of how the DWTP functions during low and high load of NoV, and potential implications for public health.