Study of Norovirus in Danish Mussels and Oysters

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Study of Norovirus in Danish Mussels and Oysters

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Conclusions: NoV was found in Danish mussels and oysters at several occasions, albeit mostly in low copy numbers. Some of the findings were from production areas of class A, which allows for placing on the market for direct consumption. Legally harvested Danish shellfish has never been linked to gastroenteritis, suggesting that the findings may not be due to infectious virus particles.

The study: **The content of Norovirus (NoV) in Danish mussels and oysters was investigated in a pilot study during 2010. The study covered 75 samples of blue mussels (*Mytilus edulis*) and 20 samples of oysters (*Ostrea edulis*). The mussels were sampled on five days spread over the year from a total of 17 production areas. One to five samples were taken in each production area, and each production area was only sampled on one sampling day. The oysters were sampled in April from four adjacent production areas. The amount of detectable genome copies was quantified by PCR as described elsewhere[1, 2].**

Table 1: Findings of NoV on sampling days as indicated. Each row gives results from one production area. One to five samples were taken from each area. No NoV was found in samples left empty.

Sampling date 2010	Class	NoV copy numbers/g DT (GG1+GGII) in sample[1]				
		1	2	3	4	5
14/4	A	0 + 6	0 + 14	15 + 18		
14/4	A	0 + 5	13 + 10	79 + 15		
14/4	B	69 + 21				
14/4	U[2]	103 + 21	124 + 26			
28/4*	A					
28/4*	A	34 + 10				
28/4*	A					
28/4*	A	52 + 1				
16/6	A/A/A[3]	119 + 2				
16/6	A/U/U[4]					
16/6	A/U/U/U[4]					
15/9	B	739 + 24	568 + 43	468 + 9	19 + 10	1728 + 35
15/9	A					
13/10	U[2]					
13/10	A					
13/10	B					
13/10	A					
1/12	A	915 + 31	139 + 29	1659 + 44	815 + 262	2047 + 129
1/12	B	182 + 12	370 + 19	1245 + 18	1518 + 8	1350 + 34
1/12	A	253 + 0	0 + 5	70 + 4	95 + 18	
1/12	A	13 + 18	1190 + 39	923 + 23		

*: Oyster samples
[1]: Theoretical limit of quantification is 50 copies/g DT. Results below this limit are only approximate
[2]: Unclassified due to inadequate number of samples
[3]: Lines classified as A in an unclassified area (Class U)
[4]: Lines classified as A or unclassified (U) due to inadequate number of samples in a class "U" area

Results: **In total, NoV was found in 34 samples (36%) originating from 12 (57%) of the tested production areas (table 1). In most cases (29 samples), the positive samples contained both genogroup (GG) I and II, but in one sample, only GGI was found, and in another four samples, only GGII was found. Two of the 20 samples of oysters were positive for both GGs, while all other positive samples were blue mussels. All samples were negative for Hepatitis A virus.**

Eight of the 12 NoV positive production areas were classified as being of class A (*E. coli* < 230 MPN/100g) This means that mussels and oysters from these areas with no further treatment can be placed on the market for direct human consumption. NoV was also found in some areas classified as B or U (unclassified) and there was no clear trend towards lower distribution and levels of NoV in class A areas compared to B or U areas. This may be due to the limited amount of data.

No temporal patterns were evident as NoV was found in samples from five out of six sampling days. However, the highest numbers of NoV were found in the December sampling, which corresponds to the winter peak normally found. Also heavy rain falls were reported prior to two of the sampling days (15/9 and 1/12) which may have caused sewage overflow and outlet to some of the production areas.

In several occasions, NoV was found to be unevenly distributed within a production area with only a few samples being weakly positive. On the other hand, there was also a high fraction of samples being positive in relatively high levels within some production areas.

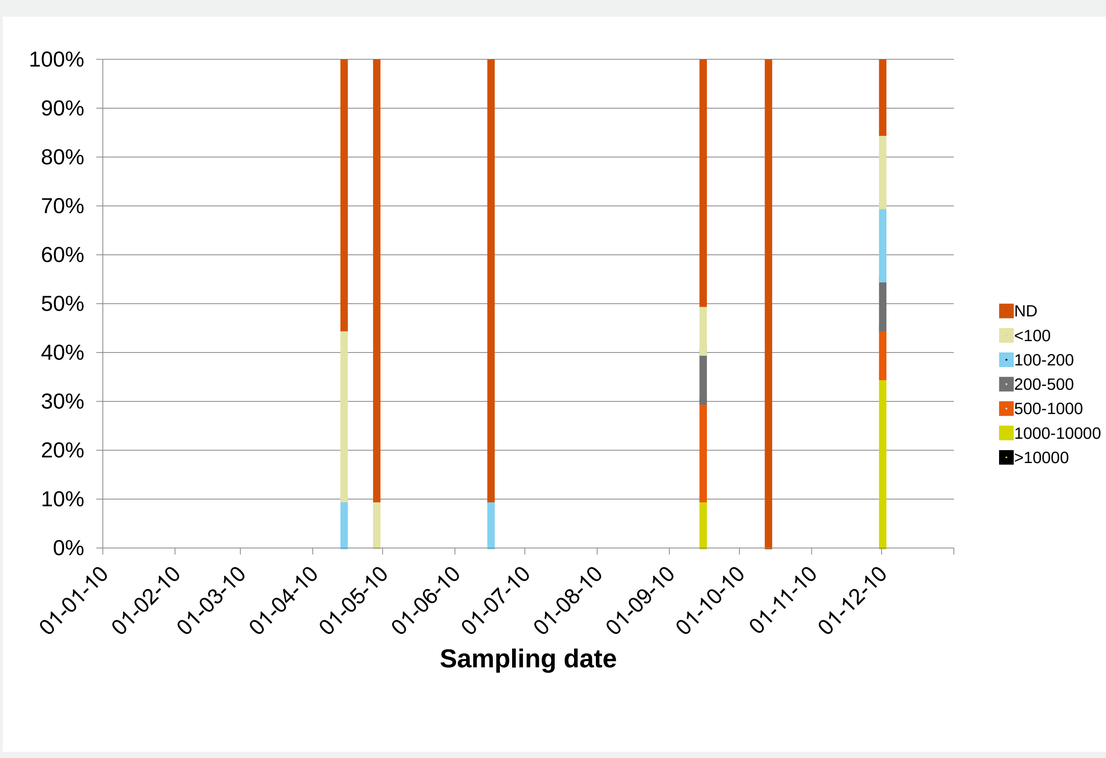

Figure 1: Fractions of samples containing NoV copy numbers within specified intervals

Discussion: **This is the first data on NoV levels in Danish oysters and mussels. Due to the "one-occasion-sampling" we cannot conclude nor exclude that these data are representative of NoV occurrence in Danish shellfish. It is noteworthy that no outbreak of human gastroenteritis has ever been attributed to consumption of legally harvested Danish mussels or oysters, although the number of virus copies found in this study is not far beyond levels recently found in imported oysters causing outbreaks. These oysters contained 118-8891 copies/g DT GGI or 9-251 copies/g DT for GGII (unplublished), but the NoV copies found may differ qualitatively from the NoV copies found in this study in regard to infectivity. Also the highest copy numbers found in this study were from mussels. In contrast to oysters, mussels are normally heat treated before consumption.**

NoV genetic material detected in mussels and oysters by PCR is not necessarily present in the form of infectious virus particles. It is thus difficult to conclude on the impact of the findings in the present study. This highlights the need for research to establish the relationship between detection of NoV in oysters by PCR and human health consequences as well as for establishing an acceptable level for NoV in oysters as pointed out recently by EFSA[(3)]. The fractions of samples from this study containing NoV copy numbers within certain intervals are presented in figure 1. This illustrates the fractions of shellfish that might be rejected at different levels.

References: **[1)] Le Guyader et al., 2009. Appl. Environ. Microbiol. 75:618-624; [2)] Uhrbrand et al., 2010. J. Virol. Methods 169:70-78; [3)] EFSA Journal 2012; 10(1)2500**

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