



# Quantitative levels of norovirus and hepatitis A virus in bivalve molluscs collected along the food chain in the Netherlands

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## Background

Contamination of bivalve molluscs with viruses is well recognized as a food safety risk. A microbiological criterion for norovirus (NoV) and hepatitis A virus (HAV) in shellfish, however, does not exist in the European Union currently.

## Objective

The aim of this study was to evaluate the contamination levels of these viruses for fluctuation over a long period (2013-2017) in oyster (n = 266) and mussel samples (n = 490) using a method based on ISO/TS 15216-1: 2013. Samples were taken at different points in the food chain, either directly post-harvest, at Dutch dispatch centers or in retail stores, from September until March of each year.

## Results

**Table:** 53.1% of the mussel and 31.6% of the oyster samples tested positive for NoV RNA. Simultaneous presence of NoV GI and GII RNA was observed in 31.6% of mussel and 10.2% of oyster samples.

	Year	No.	NoV positive <sup>a</sup>			Total (%)
			GI (%)	GII (%)	GI + GII (%)	
<b>NoV qualitative analyses in mussels</b>						
Post-harvest (A)	2015	14	7 (50)	4 (28.6)	3 (21.4)	8 (57.1)
	2016	24	4 (16.7)	5 (20.8)	1 (4.2)	8 (33.3)
	Sumc	38	11 (28.9)	9 (23.7)	4 (10.5)	16 (42.1)
Post-harvest (B)	2015	39	31 (79.5)	32 (82.1)	29 (74.4)	34 (87.2)
	2016	42	27 (64.3)	39 (92.9)	27 (64.3)	39 (92.9)
	Sumc	81	58 (71.6)	71 (87.7)	56 (69.1)	73 (90.1)
Dispatch centers	2013	60	19 (31.7)	20 (33.3)	16 (26.7)	23 (38.3)
	2014	74	25 (33.8)	18 (24.3)	14 (18.9)	29 (39.2)
	2015	44	20 (45.5)	19 (43.2)	18 (40.9)	21 (47.7)
Retail	2016	31	12 (38.7)	19 (61.3)	12 (38.7)	19 (61.3)
	2017	38	8 (21.1)	23 (60.5)	7 (18.4)	23 (60.5)
	Sumc	247	84 (34)	99 (40.1)	67 (27.1)	115 (46.6)
Total	2013	38	4 (10.5)	0 (0)	0 (0)	4 (10.5)
	2015	33	11 (33.3)	13 (39.4)	9 (27.3)	15 (45.5)
	2016	30	10 (33.3)	21 (70)	9 (30)	21 (70)
Total	2017	23	11 (47.8)	14 (60.9)	10 (43.5)	16 (69.6)
	Sumc	124	36 (29)	48 (38.7)	28 (22.6)	56 (45.2)
	490	189 (38.6)	227 (46.3)	155 (31.6)	260 (53.1)	
<b>NoV qualitative analyses in oysters</b>						
Post-harvest (A)	2015	5	2 (40)	0 (0)	0 (0)	2 (40)
	2016	3	0 (0)	1 (33.3)	0 (0)	1 (33.3)
	Sumc	8	2 (25)	1 (12.5)	0 (0)	3 (37.5)
Post-harvest (B)	2015	2	2 (100)	1 (50)	1 (50)	2 (100)
	2016	3	1 (33.3)	1 (33.3)	1 (33.3)	1 (33.3)
	Sumc	5	3 (60)	2 (40)	2 (40)	3 (60)
Dispatch centers	2013	38	8 (21.1)	5 (13.2)	2 (5.3)	11 (28.9)
	2014	66	8 (12.1)	12 (18.2)	7 (10.6)	13 (19.7)
	2015	32	9 (28.1)	1 (3.1)	1 (3.1)	9 (28.1)
Retail	2016	43	12 (27.9)	23 (53.5)	8 (18.6)	27 (62.8)
	2017	32	1 (3.1)	6 (18.8)	0 (0)	7 (21.9)
	Sumc	211	38 (18)	47 (22.5)	18 (8.5)	67 (31.6)
Total	2016	17	2 (11.8)	3 (17.6)	2 (11.8)	3 (17.6)
	2017	23	5 (20)	8 (32)	5 (20)	8 (32)
	Sumc	42	7 (16.7)	11 (26.2)	7 (16.7)	11 (26.2)
Total	2015	266	90 (18.8)	61 (22.9)	27 (10.2)	84 (31.6)

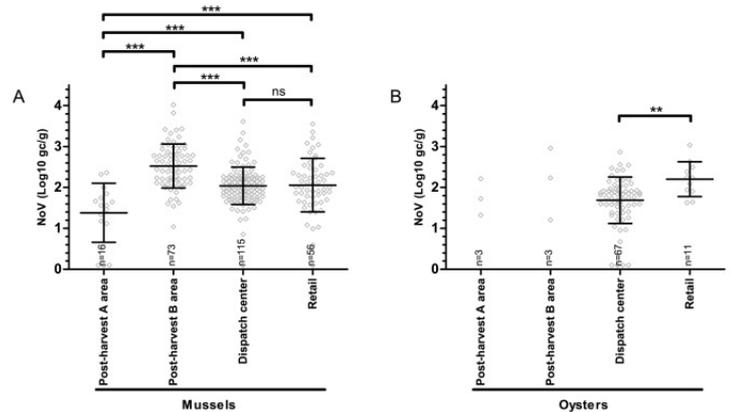
HAV RNA was detected in only one of the tested mussel samples (n = 392) (typed HAV 1A) and in none of the tested oyster samples (n = 228).

**Fig1:** Contamination levels in NoV positive mussel samples collected post-harvest from B-areas were significantly higher than in those collected post-harvest from A-areas, or at dispatch centers or retail stores. Levels in oysters from dispatch were significantly lower than those collected in retail stores. Ready for sale mussels and oysters contained 2.04 and 1.76 mean log10 transformed NoV genome copies/gram.

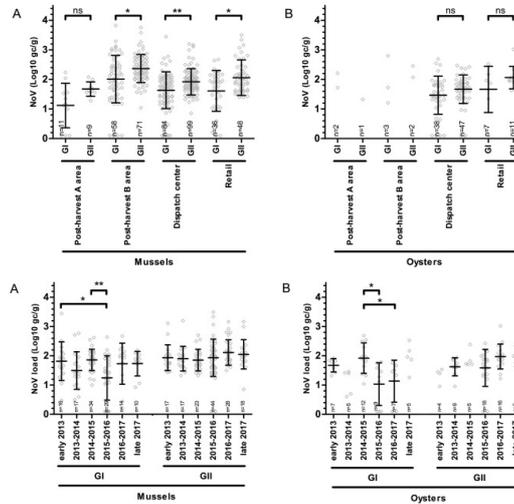
**Fig2:** NoV GII levels in mussels were higher compared to NoV GI.

**Fig3:** NoV GII levels were at a constant level in ready for sale mussels throughout all sampling periods in the study. This seemed to be true for oysters as well.

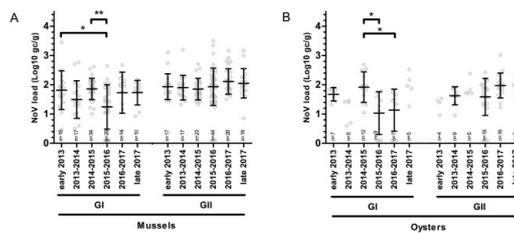
## Results



**Figure 1.** NoV contamination levels in mussel (A) and oyster (B) samples.



**Figure 2.** NoV GI and NoV GII contamination levels in mussel (A) and oyster (B) samples.



**Figure 3.** NoV GI and GII contamination levels in mussel (A) and oyster (B) samples grouped per yearly NoV winter season (September-March).

## Conclusions

- Samples analyzed for NoV (n = 756) and HAV (n = 620) RNA according to ISO/TS 15216-1:2013.
- NoV RNA detected in 53.1% of mussel and 31.6% of oyster samples, HAV once (0.3%).
- The NoV level in contaminated ready for sale oyster samples was 1.8 mean log10 genome copies/gram tissue.
- The NoV level in contaminated ready for sale mussel samples was 2.0 mean log10 genome copies/gram tissue.
- NoV GII levels were constant throughout the winters of 2013–2017.

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