Rijksinstituut voor Volksgezondheid en Milieu

Norovirus Molecular Platform

A. van Leeuwenhoeklaan 9
3721 MA Bilthoven
Postbus 1
3720 BA Bilthoven
www.rivm.nl

T 030 274 91 11
F 030 274 29 71
info@rivm.nl

Noronet report, April 2014

Janko van Beek, Annelies Kroneman, Harry Vennema, Marion Koopmans

National Institute for Public Health and the Environment, Bilthoven, The Netherlands http://www.noronet.nl / noronet@rivm.nl

The major aim of Noronet is to share epidemiological and molecular data of norovirus outbreaks in order to detect trends and identify possible (foodborne) outbreaks. This report shows the reported sequence diversity and trend between 2011 and Q1 of 2014. Data submitted between 01-01-2011 and 23-4-2014 and with sample date 2011 -2014 was used for this report. Results are shown based on sample date and the shown genotyping results are according to the norovirus typing tool (Kroneman, Vennema et al. 2011). Shown results are based on number of reported sequences. Reported outbreaks with multiple sequences were separated before analysis. Sequences that overlap the ORF1/ORF2 region are used for both ORF1 and ORF2 analysis.

Fourteen countries submitted 1363 ORF1 + 748 ORF2 sequences in 2013, of which 169 ORF1 + 210 ORF2 sequences were collected by countries in the Southern Hemisphere (Australia, New Zealand and South Africa) (table 1).

Table 1 Reported ORF1 and ORF2 sequences per country and sample year

Country	2011		2012		2013		2014		Total	
	ORF1	ORF2	ORF1	ORF2	ORF1	ORF2	ORF1	ORF2	ORF1	ORF2
AUSTRALIA	0	0	2	2	0	30	0	0	**2**	**32**
AUSTRIA	32	0	18	2	0	0	0	0	**50**	**2**
BELGIUM	6	6	17	16	52	24	0	0	**75**	**46**
CHILE	0	0	4	4	0	0	0	0	**4**	**4**
CHINA	0	77	0	0	0	0	0	0	**0**	**77**
DENMARK	15	18	23	22	14	19	0	0	**52**	**59**
FINLAND	94	0	67	0	27	15	0	0	**188**	**15**
FRANCE	184	209	178	213	236	283	66	98	**664**	**803**
GERMANY	50	59	67	67	21	20	0	0	**138**	**146**
HONG KONG	0	0	2	3	0	1	0	4	**2**	**8**
HUNGARY	82	1	47	0	53	1	2	0	**184**	**2**
IRELAND	0	0	0	4	0	0	0	0	**0**	**4**
ITALY	7	6	21	29	81	75	4	0	**113**	**110**
JAPAN	0	24	1	84	0	38	0	7	**1**	**153**
NETHERLANDS	494	24	520	3	710	26	167	67	**1891**	**120**
NEW ZEALAND	0	0	206	215	169	176	23	29	**398**	**420**
RUSSIA	0	7	0	22	0	36	2	10	**2**	**75**
SLOVENIA	0	12	0	19	0	0	0	0	**0**	**31**
SOUTH AFRICA	1	53	0	73	0	4	0	0	**1**	**130**
SPAIN	4	41	0	32	0	0	0	0	**4**	**73**
SWEDEN	4	68	6	30	0	0	0	0	**10**	**98**
UK	0	6	0	0	0	0	0	0	**0**	**6**
Total	**973**	**611**	**1179**	**840**	**1363**	**748**	**264**	**215**	**3779**	**2414**

Table 2A NoV GI diversity and trends ORF1

ORF1	2011	2012	2013	2014	Total
GI.P1	2	0	4	0	**6**
GI.P2	3	5	2	3	**13**
GI.P3	3	5	20	5	**33**
GI.P4	21	14	38	0	**73**
GI.P5	0	0	6	0	**6**
GI.P6	0	0	3	0	**3**
GI.P7	2	5	4	0	**11**
GI.P8	0	0	1	0	**1**
GI.P9	0	0	4	2	**6**
GI.Pa	1	2	2	0	**5**
GI.Pb	25	50	41	7	**123**
GI.Pd	1	1	3	0	**5**
GI.Pf	0	6	1	0	**7**
Could not assign	7	10	13	1	**31**
Total	**65**	**98**	**142**	**18**	**323**

Table 2B NoV GI diversity and trends ORF2

ORF2	2011	2012	2013	2014	Total
GI.1	3	0	5	0	**8**
GI.2	2	7	4	3	**16**
GI.3	8	22	43	7	**80**
GI.4	18	13	34	2	**67**
GI.5	0	0	7	0	**7**
GI.6	11	22	25	6	**64**
GI.7	2	9	1	0	**12**
GI.9	0	1	5	2	**8**
Total	**44**	**74**	**124**	**20**	**262**

1 Diversity and trends GI, GII and GIV

Nine percent of total reported sequences belong to GI. The most commonly detected GI genotypes are GI.Pb, GI.P4 and GI.P3 (typing based on (partial) ORF1 sequences) and GI.3, GI.4 and GI.6 (typing based on (partial) ORF2 sequences). GI.P3 and GI.P4 are elevated in 2013 compared to earlier years (table 2). The elevation in GI.P3 can be allocated to 16 reported sequences by New Zealand in 2013 and 2014. Other countries reported GI.P3 only incidentally between 2011 and 2014: Denmark, Germany and the Netherlands respectively 5, 4 and 8 sequences. The same pattern for GI.P4: New Zealand reported 24 GI.P4 sequences with sample date only in 2013, while European countries reported 49 sequences GI.P4 dispersed from 2011 until 2013.

Ninety-one percent of total sequences belong to GII. The most commonly detected GII genotypes are: GII.P4, GII.Pe, GII.P7 (ORF1) and GII.4, GII.6, GII.3 (ORF2) (table 3). We reported earlier on the emergence of the GII.Pg genotype since the norovirus season 2009/2010. GII.Pg is one of the so-called orphan genotypes without a known original associated capsid type and is found with the capsid of two other genotypes: GII.1 and GII.12. Genotype GII.Pg has been anecdotally reported in Europe between 1999 and 2007, except for a cluster of 14 outbreaks in the Netherlands in Q1 of 2001. In 2008 and 2009, GII.Pg has been occasionally reported by six countries (Denmark, Finland, France, Germany, Hungary and Netherlands), followed by a sharp increase in 2010: 93 sequences reported by ten European countries. The GII.Pg epidemic continued in 2011 and 2012 with respectively 61 and 47 reported sequences, and has been declined further in 2013.

GII.Pe has deposed GII.P4 in 2013: as far as we know this is the first year that GII.Pe is more prevalent than GII.P4. This is related to the emergence of the GII.4 Sydney_2012 ORF2 variant, which replaced the existing established GII.4 New_Orleans_2009 ORF2 variant in 2013 (figure 1). The GII.4 Sydney_2012 capsid is found mostly in combination with the GII.Pe polymerase, but occasionally uses the GII.4 New_Orleans_2009 polymerase as well. Although GII.4 has been detected in each month of 2013, it clearly peaked in January 2013 (figure 1). Not all countries were able to already submit data for the season of this year, but with the current data set GII.4 Sydney_2012 peaked in December 2013. In the southern hemisphere countries which reported in 2013 (Australia, New Zealand and South Africa), a slightly different Sydney_2012 pattern is seen, compared to the northern hemisphere countries. Australia reported most Sydney_2012 variant sequences with sample date in September 2013, while New Zealand showed a Sydney_2012 variant season from August 2012 until January 2013. South Africa reported 4 sequences with sample date in 2013 and none of them belonged to GII.4.

Besides norovirus Genogroup I and Genogroup II sequences, Germany and the Netherlands both reported a GIV sequence in respectively 2012 and November 2013. The GIV outbreak in the Netherlands affected 6 persons of which one sample was tested. This sample was obtained from a female born in 1920.

Table 3A NoV GII diversity and trends ORF1

ORF1	2011	2012	2013	2014	Total
GII.P2	11	17	35	4	**67**
GII.P3	0	1	0	0	**1**
GII.P4	722	618	299	62	**1701**
GII.P6	1	2	0	0	**3**
GII.P7	53	75	79	13	**220**
GII.P8	0	1	1	0	**2**
GII.P12	0	7	0	0	**7**
GII.P13	0	0	0	2	**2**
GII.P15	0	1	0	0	**1**
GII.P16	5	18	18	2	**43**
GII.P21	32	30	73	21	**156**
GII.P22	0	3	15	0	**18**
GII.Pc	0	0	4	0	**4**
GII.Pe	7	231	676	137	**1051**
GII.Pg	61	47	12	4	**124**
Could not assign	16	30	9	1	**56**
Total	**908**	**1081**	**1221**	**246**	**3456**

Table 3B NoV GII diversity and trends ORF2

ORF2	2011	2012	2013	2014	Total
GII.1	29	35	11	0	**75**
GII.2	15	11	26	8	**60**
GII.3	45	29	16	11	**101**
GII.4	402	571	484	145	**1602**
GII.5	0	4	14	0	**18**
GII.6	42	70	25	25	**162**
GII.7	18	26	28	0	**72**
GII.8	0	1	0	0	**1**
GII.10	0	2	0	0	**2**
GII.12	4	6	1	1	**12**
GII.13	6	5	10	2	**23**
GII.14	2	2	6	1	**11**
GII.15	0	0	1	0	**1**
GII.16	1	1	0	0	**2**
GII.17	1	0	0	0	**1**
GII.21	2	3	0	0	**5**
Could not assign	0	0	2	2	**4**
Total	**567**	**766**	**624**	**195**	**2152**

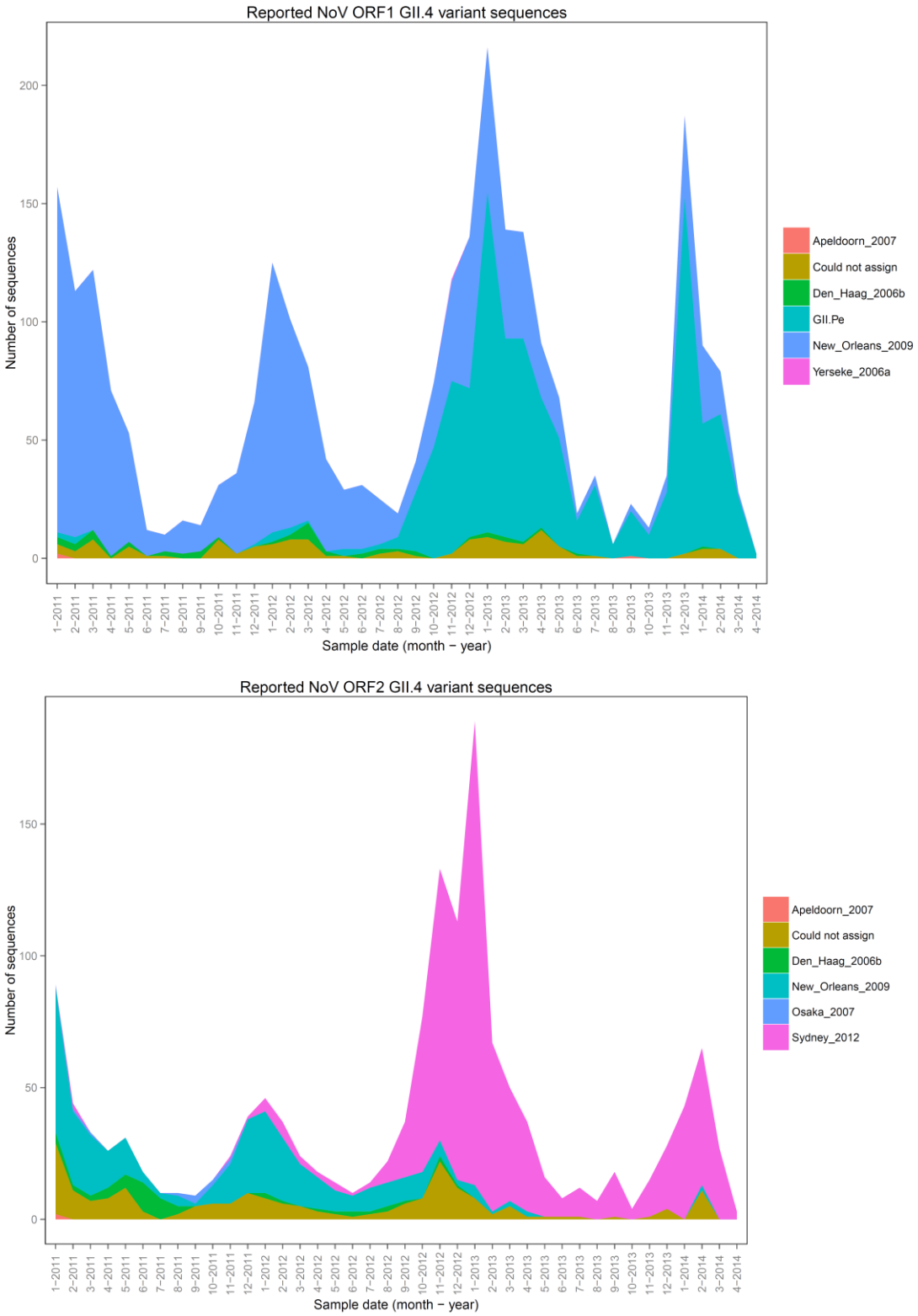

Figure 1 GII.P4 variant + GII.Pe distribution by sample month and year based on ORF1 (top) and ORF2 (bottom) sequences (stacked plots).

2 Capsid enhanced surveillance

Last year, we proposed to start enhanced GII.4 capsid surveillance in order to be able to detect new GII.4 variants in an early stage. Eight countries showed interest to participate in the project: Canada, India, Finland, Hungary, Hong Kong, Netherlands, New Zealand and Slovenia. Participating labs are asked to sequence a (partial) capsid sequence from 10 randomly chosen GII.4 samples each quarter and upload the results to the noronet database. Next month we will send a protocol, used at RIVM, for sequencing of ORF2, including the P2 domain. We would like to start this year summer, sequencing 10 GII.4 samples for Q3 2014. If you are not on the list and would like to participate in the project, please send us an email.

3 NoV typing

Over the last couple of years we observed an increase in the number of PCR positive samples that were negative in the typing PCR. This indicated that the typing PCR primers needed to be updated. We have good experience with so called CODEHOP primers for enterovirus and hepatitis E virus typing. The high variability of norovirus is condition that would favor a similar approach for norovirus. Development of these primers proved to be challenging for the whole genus norovirus but it was feasible at the species or group level. We developed nested PCRs for GI and for GII norovirus for the fragment from region A to region C. It is a fragment of 1200 nucleotides in length in the first PCR. A fragment of 1100nt from the second PCR can be sequenced from both ends with specific sequence primers. Sequencing yields a sequence of on average 1000nt of which about 80% is double stranded. It contains information on both ORF1 and ORF2 and therefore also on whether or not a strain is a recombinant. The ORF2 part of the fragment is roughly equivalent to region C and can provide information on both the genotype and the variant in case of GII.4. The fragment also contains the JV13i primer binding site. In some genotypes such as GII.16 and GII.7 and several GI types the binding site for the JV13i primer contains several changes (figure 2 bottom half). We assume that this is the reason we failed to detect these with the old typing PCR. Some other samples that failed in the old typing method had no changes in the JV13i binding region but all had high Ct values (figure 2 top half). This suggests

that the new typing protocol is also more sensitive than the old protocol.
The protocol is going through the final stages of validation and will be made available soon.

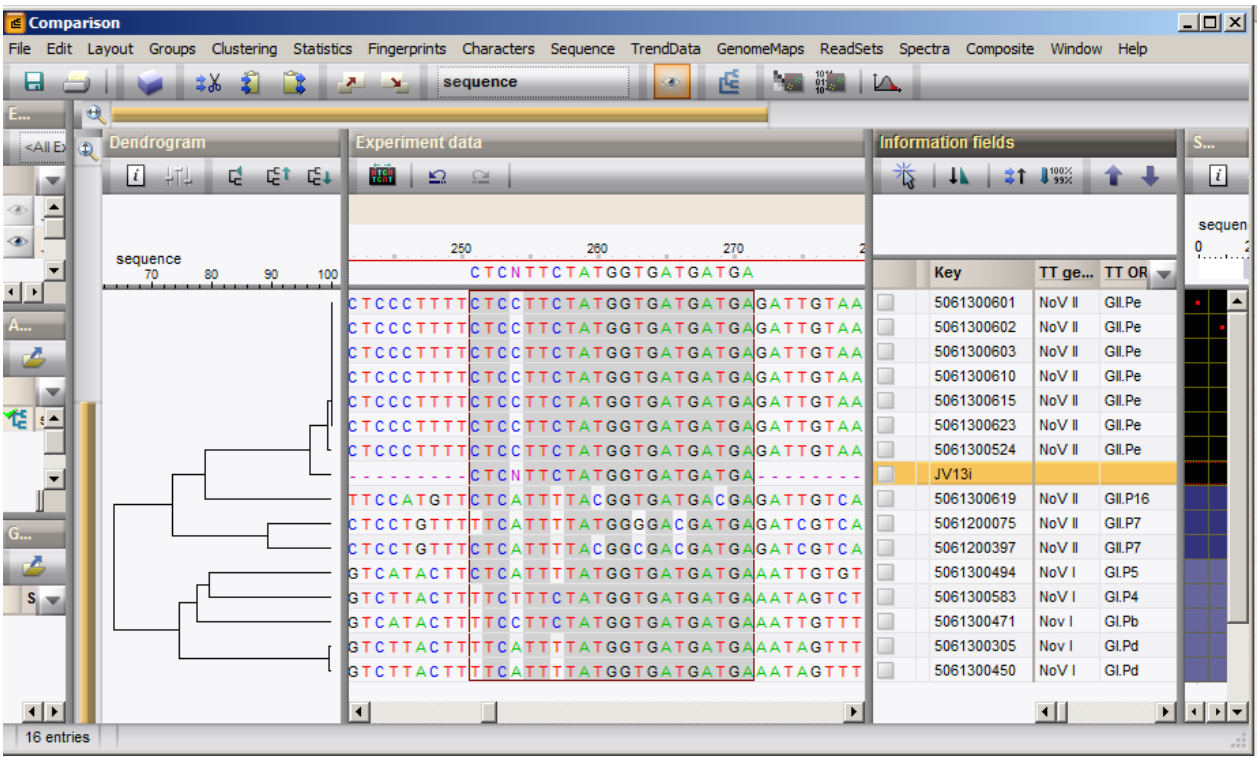

Figure 2 Sequence diversity among various NoV genotypes at JV13i primer binding site

4 Literature

Kroneman, A., H. Vennema, et al. (2011). "An automated genotyping tool for enteroviruses and noroviruses." J Clin Virol 51(2): 121-125.