

Emergence of a New Norovirus Variant in Scotland in 2006[∇]

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For each month between January 2005 and August 2006, a representative number of outbreaks was examined using nucleic acid sequence analysis. Using this method, we showed that an increase in norovirus activity coincided with the emergence of a new GII genotype 4 variant, which by March 2006 was detected in the majority of health boards in Scotland.

Data from the West of Scotland Specialist Virology Centre (WOSSVC) in Glasgow has shown there to be a large increase in norovirus activity (detections and outbreak detections) in Scotland in the first quarter of 2006. This increase was so large that it contributed to making 2006 a year of more norovirus detections than any of the previous years since norovirus reverse transcription-PCR testing has been routinely performed. This increase in activity was also detected by national surveillance systems and was shown to be nationwide (5). The increase could not be linked to any major change in laboratory or healthcare procedure that may have resulted in such a large increase in norovirus cases. In anticipation that this increase in norovirus activity was due to the emergence of a new norovirus variant, sequence analysis of the RdRp and the adjacent region of *orf2* was carried out for a representative number of norovirus outbreaks diagnosed at our laboratory for each month between January 2005 and August 2006.

Between these dates, a total of 331 outbreaks were detected by the WOSSVC. All outbreaks were confirmed as having been caused by GII noroviruses using a dual-labeled, probe-based, real-time PCR and were stored at 4°C after submission to the laboratory (3). Sequencing was attempted on 175 samples. Each sample was taken from a different outbreak during the study period and represented between 26 and 100% of the total outbreaks occurring for each month of the study period.

Sequencing was successful in 160 of the 175 samples. In total, 149 of the 160 samples analyzed were classified as being members of GII genotype 4 (GII-4). The remaining 11 samples had similar amino acid sequences, and sequence analysis showed that their sequences were very similar to those of “Groundwater in A hotel (Noro-GII-Water-A) (DQ004657)” (7) and outbreak 03048 (AB186103) (11). Kim et al. (7) classified the virus as a GII-3 norovirus, while Seto et al. (11) described the virus as a GII-6 norovirus. Since these samples were not shown to be the predominant or emerging strains during the period of study, they were excluded from further analysis.

The 149 GII-4 strains were assigned to a variant group according to conserved nucleotides at positions 18 (A or G),

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TABLE 1. Norovirus variants

Variant	Amino acid motif
v1	N ₆ S ₉ T ₁₅
v2	N ₆ N ₉ T ₁₅
v3	N ₆ T ₉ T ₁₅
v4	S ₆ N ₉ T ₁₅
v5	S ₆ T ₉ T ₁₅
v6	N ₆ N ₉ A ₁₅

26 (G, A, or C), and 43 (A or G) of the *orf2* gene, as previously determined (1, 2, 8–9). These variant-defining point mutations altered amino acid motifs at positions 6, 9, and 15 as described in Table 1. Based on these criteria, the following genotype 4 variants were detected: GII-4 variant 3 (v3) or GII-4 v4, GII-4 v5, and GII-4 v6 (Table 2). By using BLAST, we found the GII-4 v3 noroviruses to be similar to

TABLE 2. Norovirus GII-4 variants detected in the study

Date	No. of outbreaks detected	No. of outbreaks tested (%)	No. of norovirus GII-4 variants detected				Not typed
			v3	v4	v5	v6	
January 2005	32	15 (46%)	11				4
February 2005	16	10 (62%)	9				1
March 2005	29	9 (31%)	8		1		
April 2005	25	8 (32%)	8				
May 2005	18	6 (33%)	3				3
June 2005	9	5 (55%)	5				
July 2005	3	0					
August 2005	4	3 (75%)	3				
September 2005	5	3 (60%)	2				1
October 2005	3	1 (33%)	1				
November 2005	6	2 (33%)	1				1
December 2005	15	5 (33%)	4				1
January 2006	21	7 (33%)	6	1			
February 2006	18	10 (55%)	8	1			
March 2006	49	36 (73%)	11	24		1	
April 2006	36	22 (61%)	3	19			
May 2006	23	6 (26%)	1	5			
June 2006	10	5 (50%)		5			
July 2006	3	3 (100%)		3			
August 2006	7	5 (71%)		5			
Total	332	160 (48%)	84	63	1	1	11

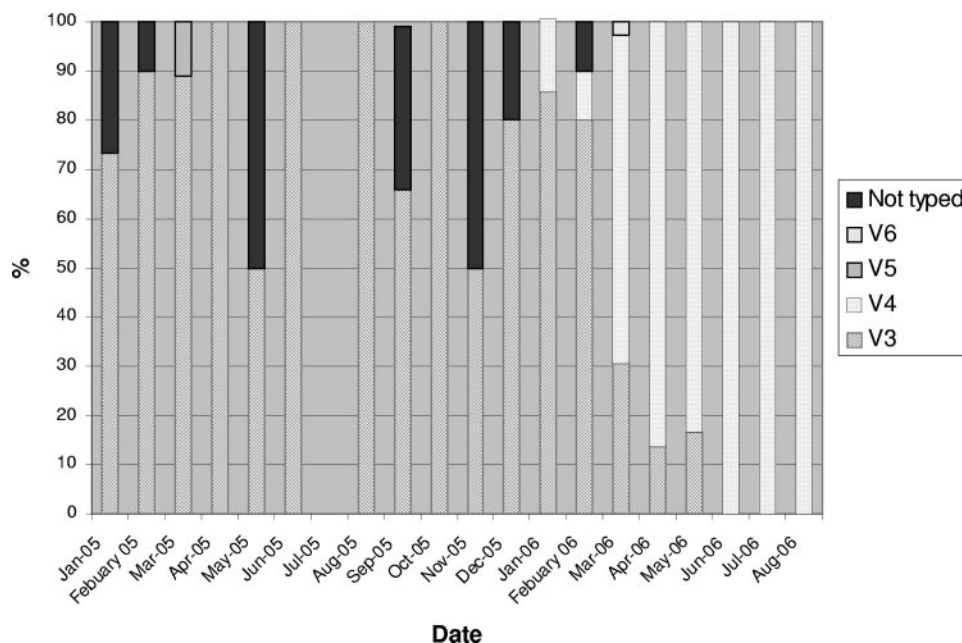


FIG. 1. Detection rate (%) of norovirus variants per month.

norovirus Hu/NoV/414055/2004/uk (DQ676863). The v4 noroviruses were similar to norovirus Hu/GII-4/Rhyl440/2005/UK (DQ665819). The single v5 norovirus was similar to norovirus Hu/GII-4/Portsmouth/2004/UK (DQ676864), while the v6 norovirus was similar to norovirus Hu/GII-4/Lincoln House/2006/UK (DQ822045).

An examination of the distribution of the detected norovirus variants over time highlights that the increase in norovirus outbreak activity observed in Scotland in 2006 coincided with the emergence of a new GII-4 v4, which became the predominant strain by March 2006. For example, of the 84 samples that were analyzed from outbreaks occurring between January 2005 and February 2006, 69 (82%) were GII-4 v3. Conversely, of the 77 samples that were analyzed from outbreaks occurring from March 2006 onwards, 61 (79%) were GII-4 v4 (Fig. 1).

The earliest example of norovirus GII-4 v4 occurring in the area under study was from a sample taken at Gartnavel Hospital, Glasgow, United Kingdom, on 5 January 2006 (this hospital is part of Greater Glasgow Health Board). The only other sample taken before 9 March 2006 that contained GII-4 v4 was taken in Hairmyres Hospital, East Kilbride, United Kingdom, on 21 February 2006 (Lanarkshire Health Board). From March 2006 onwards, the GII v4 norovirus was detected in samples from 9 of the 15 recognized health boards in Scotland and, as a result, its emergence could be considered to be national and not restricted to local health boards. The same variant was shown to emerge from England in January 2006 and Wales in 2006 (2). This emergence was also associated with a large increase of reported norovirus cases and a prolonged norovirus season, with cases occurring in June and July.

Previous studies have also described the emergence of a new variant, eventually replacing the predominant strain (2, 4, 8). Evidence suggests that this emergence of a new variant occurs every 2 to 3 years and is often associated with an increase in attack rates. At present, the reasons why this increase occurs

are unclear but it is thought to likely be a result of a complex relationship between viral and host factors. For example, perhaps the new variant is more virulent, is able to evade the immune system, has a replicate advantage, or is more environmentally stable than other noroviruses. Recent data have highlighted the link between various genetic factors and norovirus infectivity (6, 10). This link may explain the worldwide predominance of GII-4 norovirus types. Unlike some norovirus types, these viruses have been shown to be able to bind to a broad range of carbohydrate targets. As a result, they have a large susceptible target population.

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