Arch Virol (2007) 152: 1295–1303
DOI 10.1007/s00705-007-0954-9
Printed in The Netherlands

Archives of Virology

Inter-seasonal diversity of norovirus genotypes: Emergence and selection of virus variants

C. I. Gallimore, M. Iturriza-Gomara, J. Xerry, J. Adigwe, and **J. J. Gray**

Centre for Infections, Health Protection Agency, Enteric Virus Unit, Virus Reference Department, Colindale, London, U.K.

Received August 9, 2006; accepted February 7, 2007; published online March 15, 2007

Summary

This study describes a method used to determine the diversity of NoVs co-circulating in the community that consisted of the analysis of a limited number of strains collected from outbreaks occurring at different times of the NoV season. The diversity of twenty NoV strains collected from outbreaks occurring at the beginning of each NoV season (September) was compared to the diversity found in the middle (December) and at the end of the season (March). The method was validated through the characterisation of greater numbers of strains at times when novel genotypes or variants were detected. A total of 864 strains from outbreaks of gastroenteritis from the 2003/04, 2004/05 and 2005/06 seasons were genotyped, with the majority of outbreaks occurring in the UK.

There was a greater diversity of NoV genotypes at the beginning of two of the three seasons, 2003/04 and 2005/06, when compared to strains circulating at the end of the seasons, and GII-4 NoV strains predominated (>90%) at the end of each season. Data from this study also identified the co-circulation and differentiation of three major GII-4 variants (v2, v3, and v4). Detailed analysis of a larger number of strains throughout each season confirmed that variants emerged, became the predominant circulating strain and were ultimately replaced with another variant selected from a pool of variants. By June 2006, GII-4 v4 (Hu/NoV/Rhyl440/2005/UK) emerged as the predominant GII-4 strain, usurping the previous GII-4 v3 strain [Hu/NoV/Hunter284E/040/AU] to become the commonest co-circulating strain, in the UK in 2006.

Introduction

Noroviruses (NoVs) are members of the family *Caliciviridae* [12] and are the commonest cause of outbreaks of acute gastroenteritis world-wide [16]. NoV outbreaks are frequently associated with semi-closed or closed institutions such as hospitals [11] and homes for the elderly [13]. Outbreaks also occur in other settings, including eating establishments [26], cruise ships [13], concert halls [3] and schools [18]. Transmission of NoVs is usually by person-to-person spread [17], although food [1, 7, 20], including shellfish [21], water [10], and environmental or airborne contamination have all been implicated in transmission [8, 11, 25].

Author's address: Chris I. Gallimore, Centre for Infections, Enteric Virus Unit, Virus Reference Department, Health Protection Agency, Colindale, London, NW9 5HT, U.K. e-mail: christopher.gallimore@hpa.org.uk

The genomic diversity of human NoVs includes three genogroups (GI, GII and GIV), sixteen genotypes and a number of provisional genotypes, which have yet to be formally acknowledged. Furthermore, several genetic lineages and variants can be distinguished within genotypes, some of which have epidemiological significance. Among GII-4 strains, at least six variants have been identified between 2000 and 2006, of which GII-4 v2 was found to be responsible for a widespread epidemic of gastroenteritis in 2002 [23, 24]. Diversity among NoVs is maintained through the accumulation of point mutations associated with the error-prone nature of RNA replication and genetic recombination involving the exchange of sequences between two similar RNA viruses. Several features of RNA viruses (antigenic variation, genotypic diversity, immune escape) have been attributed to the lack of proofreading of the RNA-dependent RNA polymerases [27]. This error-prone nature of template copying by RNA polymerases during virus replication [2] can lead to conserved point mutations, either silent or resulting in amino acid substitutions.

Immunological protection from NoV infection and/or disease is poorly understood. Immunity is likely to be short-lived and primarily genogroup-, if not genotype-specific [9]. Protection from infection at mucosal sites is unlikely, as the short incubation period following NoV infection is likely to overwhelm a previously primed mucosal immune response. In individuals infected within the last six to twelve months or repeatedly exposed through water sports or the regular consumption of shellfish, immunity may be cumulative and is likely to protect from symptomatic infection rather than preventing infection *per se*. The complex nature of mucosal immunity, tied to the mutability and resulting diversity of NoVs has resulted in a population of co-circulating virus genogroups, genotypes and variants causing both symptomatic and asymptomatic infection in the population [4].

This study examines the intra- and inter-seasonal diversity of NoV genotypes in England and Wales over three NoV seasons, 2003/04, 2004/05 and 2005/06, and in particular, the diversity within GII-4 strains, which demonstrated a periodic emergence of new virus variants.

Materials and methods

Specimens and NoV detection

The specimens used in this study form part of a national NoV collection. The collection was derived from outbreaks occurring each year in specific geographical areas of England and Wales. NoV was identified as the cause of gastroenteritis in 2946 outbreaks occurring in the winter seasons between September 2003 and June 2006. Initially, 180 (6.1%) strains, 20 each from the beginning (September/October), middle (December/January) and end (March/April) of each of the three seasons were characterised. A further 684 (23.2%) NoV strains of undetermined genotype were characterised in order to confirm or expand the findings from the initial 180 strains.

Strain characterisation

Nucleic acid extraction

Faecal specimens from outbreaks were prepared as 10% suspensions in Star^{TM} buffer (Roche Molecular Biosystems, Lewis, UK), and total RNA was extracted using a Total Nucleic Acid Extraction Kit (Roche Molecular Biosystems) and a MagNApure^{TM} extraction machine (Roche Molecular Biosystems).

Reverse-transcription

Extracted RNA was reverse transcribed to complementary DNA (cDNA) using MMLV and random primers as described previously [5].

NoV genotyping algorithm

The NoV genotyping algorithm comprised GI- and GII-specific hemi-nested PCR assays and sequencing (see Fig. 1). cDNAs were tested in GI and GII primary assays, and amplicons were characterised through direct sequencing. Samples that were negative in the primary PCRs were tested in a secondary set of PCRs, and amplicons were sequenced directly. Both primary and secondary PCR assays targeted the region spanning the Orf1/Orf2 (primers are described in Table 1).

Primary genotyping PCR

cDNAs were tested in primary single-round and/or primary hemi-nested PCRs GI A: GII A and GI B: GII B, respectively, using the primers and conditions described previously [6, 15] (Table 1). The GI- or GII-specific single-round PCR assays amplified regions of 597 bp and 468 bp, for GI (GI A) and GII (GII A) strains, respectively. The primary hemi-nested PCRs produced amplicons of 364 bp and 343 bp for GI (GI B) and GII (GII B) strains, respectively.

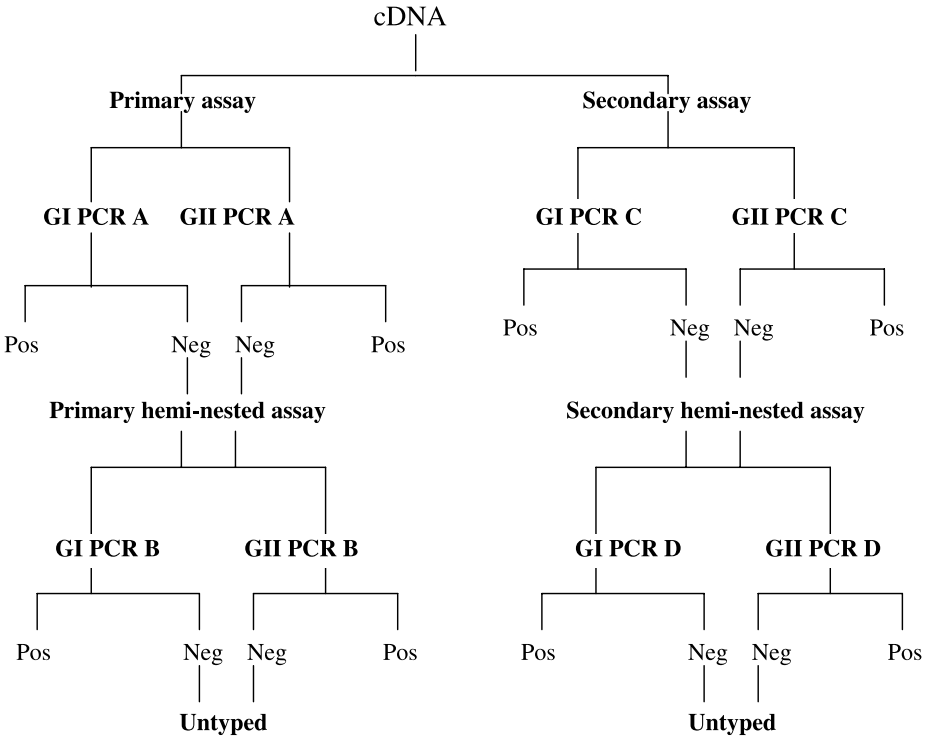

Fig. 1. Testing algorithm for NoV typing PCR. All positives (*Pos*) were sequenced, for PCR assays, see methods

Table 1. NoV genotyping PCR primer locations

PCR assay	Primer	Direction	Sequence 5′	Position (nt)	Reference
GI A	GIFF-1	forward	ath gaa cgy caa aty ttc tgg ac	5060–5082	[15]
GI A	GIFF-2	forward	ath gaa aga caa atc tac tgg ac	5060–5082	[15]
GI A	GIFF-3	forward	ath gar agr car ctn tgg tgg ac	5060–5082	[15]
GI B and C	GIFFN	forward	gga gat cgc aat ctc ctg ccc	5313–5330	[6]
GI D	GIFFN2	forward	atc tcc tgc ccg awt wyg taa	5322–5342	this study
GI A, B, C and D	GISKR	reverse	cca acc car cca ttr tac a	5637–5656	[19]
GII A	GIIFB-1	forward	ggh ccm bmd tty tac agc aa	4922–4941	[15]
GII A	GIIFB-2	forward	ggh ccm bmd tty tac aag aa	4922–4941	[15]
GII A	GIIFB-3	forward	ggh ccm bmd tty tac arn aa	4922–4941	[15]
GII B and C	GIIFBN	forward	tgg gag ggc gat cgc aat ct	5048–5067	[6]
GII D	GIIFBN2	forward	gcg atc gca atc tgg ctc cc	5054–5074	this study
GII A, B, C and D	GIISKR	reverse	ccr ccn gca trh ccr ttr tac at	5367–5389	[19]

* Lordsdale virus (X86557), # Norwalk virus (M87661).

Secondary genotyping PCR

The GI- or GII-specific single-round secondary PCR assays amplified regions of 364 bp and 343 bp for GI (GI C) and GII (GII C) strains, respectively, and the secondary hemi-nested PCRs amplified regions of 357 bp and 333 bp for GI (GI D) and GII (GII D) strains, respectively (Fig. 1, Table 1).

For the secondary PCR, 5 µl of cDNA was added to a 45 µl PCR mix containing 10 mM Tris pH 8.0, 50 mM HCl, 2.5 mM $MgCl_2$, 1 mM of each dNTP (Invitrogen, Paisley, UK), 20 pmols of each forward primer and 40 pmols of the reverse primer and 2 units of TAQ DNA polymerase (Invitrogen, UK). Products were amplified using the following

conditions: 95 °C for 2 min, then 40 cycles of 95 °C for 30 sec, 48 °C for 30 sec and 72 °C for 2 min, followed by 1 cycle of 72 °C for 5 min, then hold at 15 °C.

For the secondary hemi-nested PCR, 1 μl of first-round template was added to a 49-μl PCR mix containing 10 mM Tris pH 8.0, 50 mM HCl, 3.0 mM $MgCl_2$, 1 mM of each dNTP (Invitrogen, UK), 20 pmols of each forward primer and 40 pmols of the reverse primer and 2 units of TAQ DNA polymerase (Invitrogen, UK). Products were amplified using the following conditions: 95 °C for 2 min, then 25 cycles of 95 °C for 30 sec, 48 °C for 30 sec and 72 °C for 2 min, followed by 1 cycle of 72 °C for 5 min, then hold at 15 °C.

DNA sequencing

Purified DNA was sequenced in both directions using the Beckman Coulter™ CEQ2000 Dye Terminator Cycle Sequencing Quick Start kit (according to the manufacturer's instructions) and a Beckman Coulter™ CEQ2000 capillary sequencer. Generation of contiguous sequences and pairwise alignments of the inter-primer region, 425 bp (GIIFB-1, -2, -3)/GIISKR), 300 bp (GIIFBN/GIISKR), 273 bp (GIIFBN2/GIISKR), 555 bp (GIFF-1, -2, -3)/GISKR), 324 bp (GIFFN/GISKR), 314 bp (GIFFN2/GIISKR), was performed using Genebuilder and Clustal in Bionumerics ver. 2.5 (Applied Maths, Kortrij, Belgium).

Sequences from this study submitted to GenBank

Nucleotide and amino acid sequences derived from the 5′end Orf2 (282 bp) from GII-4 variants have been submitted to GenBank, v1 strain Hu/NoV/123027/2001/UK (DQ676861), v2 strain Hu/NoV/Brynhaven/2003/UK (DQ676862), v3 strain Hu/NoV/414055/2004/UK (DQ676863), v4 strain Hu/NoV/Rhyl440/2005/UK (DQ665819), v5 strain Hu/NoV/Portsmouth/2004/UK (DQ676864) and v6 strain Hu/NoV/Lincoln House/2006/UK (DQ676865). Nucleotide and amino acid sequences derived from a 582-bp region at the 3′end of the Orf1 of prototypes of GII-4 variants v2–v6 have also been submitted to GenBank and include v2 strain Hu/NoV/Brynhaven/2003/UK (DQ822037), v2 strain Hu/NoV/U/2004/UK (DQ822038), v3 strain Hu/NoV/414055/2004/UK (DQ822039), v4 strain Hu/NoV/Rhyl440/2005/UK (DQ822040), v4 strain Hu/NoV/614025/2006/UK (DQ822041), v4 strain Hu/NoV/Chester/2006/UK (DQ822042), v5 strain Hu/NoV/Portsmouth/2004/UK (DQ822043), v5 strain Hu/NoV/422031/2004/UK (DQ822044) and v6 strain Hu/NoV/Lincoln House/2006/UK (DQ822045).

Statistics

The χ^2 test was used to determine the significant difference observed among seasons and within seasons.

Table 2. Inter- and intra-seasonal diversity of NoV genotypes during 2003–2006

Genotype	2003/04			2004/05			2005/06		
	Early	Middle	Late	Early	Middle	Late	Early	Middle	Late
GI-1	0	0	0	0	0	0	0	1 (5%)	0
GI-2	1 (5%)	0	0	0	0	0	0	0	0
GI-3	1 (5%)	0	0	0	0	0	1 (5%)	1 (5%)	0
GI-4	0	0	0	0	0	0	0	3 (15%)	0
GI-5	0	0	0	0	0	0	1 (5%)	0	0
GI-6	2 (10%)	0	0	0	0	0	1 (5%)	0	0
GII-1	0	0	0	0	0	1 (5%)	0	1 (5%)	0
GII-2	3 (15%)	1 (5%)	0	4 (20%)	1 (5%)	0	0	1 (5%)	0
GII-3	7 (35%)	3 (15%)	2 (10%)	0	0	0	0	2 (10%)	0
GII-4	1[a] (5%)	14[b] (70%)	18[c] (90%)	14[d] (70%)	18[e] (90%)	18[f] (90%)	9[g] (45%)	7[h] (35%)	18[i] (90%)
GII-6	1 (5%)	0	0	2 (10%)	1 (5%)	0	2 (10%)	0	1 (5%)
GII-7	3 (15%)	2 (10%)	0	0	0	1 (5%)	6 (30%)	3 (15%)	1 (5%)
GII-8	1 (5%)	0	0	0	0	0	0	1 (5%)	0
Total	20	20	20	20	20	20	20	20	20
Total of genotypes	3 GI 6 GII	4 GII	2 GII	3 GII	3 GII	2 GII	3 GI 3 GII	3 GII 6 GII	3 GII

Early September/October, *Middle* December/January, *Late* March/April, *GI* Genogroup I, *GII* Genogroup II, *v* variant, *ND* variant not determined.

GII-4 variants from outbreaks are as follows: [a] v2 (1), [b] v2 (7), v3 (2), vND (5), [c] v2 (18), [d] v2 (3), v3 (5), v5 (1), vND (5), [e] v3 (18), [f] v3 (18), [g] v3 (9), [h] v2 (1), v3 (6), [i] v3 (10), v4 (8).

Results

A total of 864/2946 (29.3%) strains from outbreaks of gastroenteritis that occurred between September 2003 and June 2006 were characterised by partial sequencing of the gene encoding the virus capsid. The first 20 outbreaks occurring in the early, middle and late periods of the NoV seasons were compared for each of the three seasons (Table 2). In September 2003, a total of 9 different genotypes were co-circulating in the populations of England and Wales. As the season progressed, diversity of genotypes diminished with four genotypes detected mid-season and two at the end of the season. GII-4 strains represented 5, 70 and 90% of the circulating strains at the beginning, middle and end of the season, respectively. Significantly less genotype diversity ($P = 0.02$) was observed during the 2004/05 season, with GII-4 predominating throughout. Although GII-4 was again predominant at the beginning of the 2005/06 season, greater genotype diversity was observed mid-season, again with 9 genotypes co-circulating in the population (Table 2). Interestingly, the 2005/06 season was protracted, with significant numbers of outbreak samples referred in June and July, 2006 (data not shown). By March 2006, GII-4 strains again predominated, being associated with 90% of late season outbreaks.

The GII-4 v2 strain was detected in March 2004 and latterly in December 2005/January 2006 through sequencing a limited number of strains each

Table 3. Norovirus outbreaks during 2003–2006 and outbreaks caused by GII-4 variants

All NoVs				GII-4 Outbreaks						
Date	Outbreaks total	Outbreaks sequenced (%)	Total outbreaks sequenced as GII-4 (%)	v2	v3	v4	v5	v6	Variant determined	Variant not determined
Sep-03	74	8 (11)	1 (12)	1					1	
Oct-03	41	17 (42)	1 (6)	1					1	
Nov-03	35	7 (20)	1 (14)							1
Dec-03	35	7 (20)	6 (86)	2	1				3	3
Jan-04	46	11 (24)	7 (55)	5	1				6	1
Feb-04	52	26 (50)	13 (88)	9	1				10	3
Mar-04	118	38 (32)	11 (87)	26	1		1		28	5
Sep-04	47	13 (28)	11 (85)	3	2		1		6	5
Oct-04	61	31 (51)	22 (70)	2	18				20	2
Nov-04	157	62 (39)	53 (85)	2	44		6		52	1
Dec-04	241	44 (18)	42 (95)		39				39	3
Jan-05	277	15 (5)	13 (86)		7		1		8	5
Feb-05	240	14 (6)	12 (86)		9		1		10	2
Mar-05	162	9 (6)	7 (77)		7				7	
Sep-05	24	4 (16)	1 (25)		1				1	
Oct-05	55	28 (51)	14 (50)		10				10	4
Nov-05	39	15 (38)	7 (47)		7				7	
Dec-05	108	48 (44)	24 (50)	1	17	1			19	5
Jan-06	184	63 (34)	47 (75)	2	34	4			40	7
Feb-06	257	63 (25)	52 (82)		38	8		2	48	4
Mar-06	218	109 (50)	94 (86)		64	23		1	88	6
Apr-06	208	97 (47)	93 (96)		44	37		5	86	7
May-06	145	68 (46)	63 (86)		13	36		9	58	5
Jun-06	122	67 (55)	67 (55)		13	46	1	7	67	
Total	2946	864 (29)	684 (79)	54	371	155	11	24	615	69

GII-4 variants (v), *v2* NNT, *v3* NTT, *v4* SNT, *v5* STT and *v6* NNA, *GT* genotyped, *ND* variant not determined

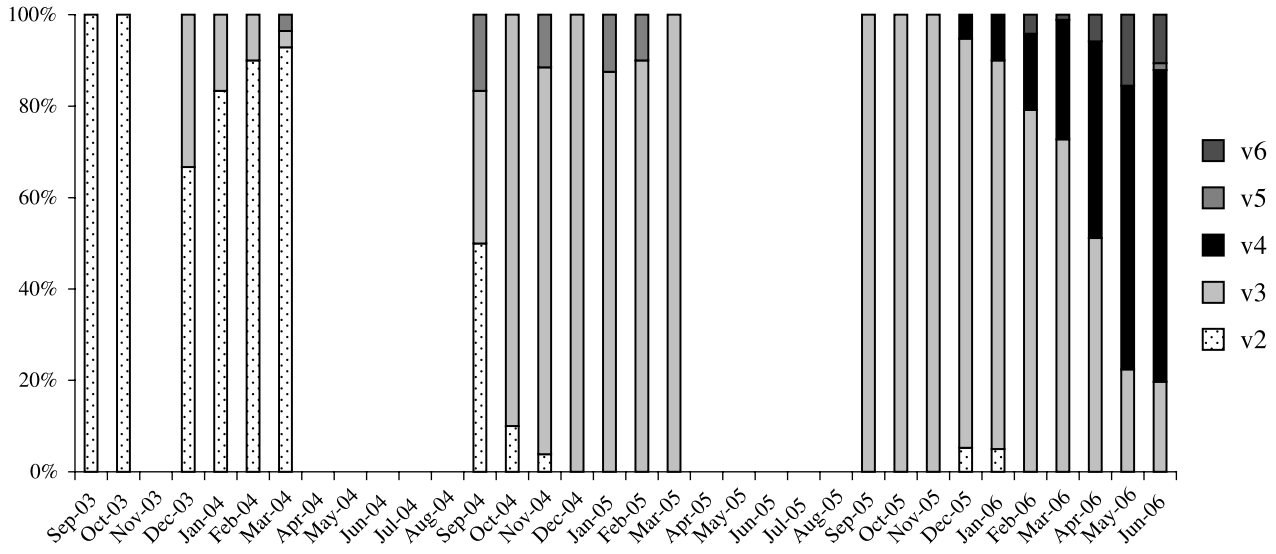

Fig. 2. Percentage distribution of GII-4 variants from September 2003–June 2006. *v2* NNT, *v3* NTT, *v4* SNT, *v5* STT and *v6* NNA, data from April to August 2004 and 2005, not included in this study

season ($n = 60$; Table 2). More extensive strain characterisation throughout each season was performed in order to more accurately identify when these variants first became detectable in the human population in England and Wales (Table 3).

The inter-seasonal diversity of 684 GII-4 strains was examined, and strains were assigned to a variant group according to conserved nucleotides at positions 18 (A or G), 26 (G, A or C) and 43 (A or G) of the gene encoding the capsid. Variants were numbered chronologically. These variant-defining point mutations encoded the following amino acid (aa) motifs at aa positions 6, 9 and 15; v2: $N_6N_9T_{15}$, v3: $N_6T_9T_{15}$, v4: $S_6N_9T_{15}$, v5: $S_6T_9T_{15}$, v6: $N_6N_9A_{15}$. GII-4 variant v1 was identified before 2003 and was characterised by the following motif; v1: $N_6S_9T_{15}$. GII-4 v2 predominated throughout the 2003/04 season, and v3 was first detected co-circulating with v2 in December 2003. Variant 3 became the predominant strain throughout the 2004/05 season and until April 2006 of the following season. GII-4 v4 was first detected in December 2005 and became the predominant strain by May 2006. Variants 5 and 6 circulated at lower levels during the 2004/05 and 2005/06 seasons, respectively, although a single outbreak associated with variant 5 was identified in June 2006 (Table 3, Fig. 2).

Discussion

This study investigated the representativeness of determining the diversity and periodicity of NoV genotypes co-circulating in outbreaks of gastroenteritis by investigating the first twenty outbreaks occurring in September/October, December/January and March/April of years 2003–2006, representing early, middle and late periods of the NoV seasons. This model identified a wide range of genotypes and variants within genotypes, particularly GII-4 variants. These results were confirmed through the characterisation of a larger number of strains circulating during periods when a novel strain or new variant was identified. GII-4 variants v2, v3 and v5 were identified in each of the 60 samples from the 2003/04, 2004/05 and 2005/06 seasons. Although only GII-4 v3 and v4 were detected after initial screening of 60 samples from the 2005/06 season, v2 and v6 were identified after more extensive strain characterisation but were circulating at very low levels compared with GII-4 v3 and v4, which predominated during the 2005/06 season.

A wide diversity of genotypes was identified early in the 2003/04 season. This followed the introduction into the population of a previously reported variant (v2) of GII-4 [23, 24]. GII-4 v2 was epidemic

in 2002, and widespread infection may have resulted in a high level of strain-specific short-term herd immunity. This would account for the low incidence of symptomatic infections with GII-4 strains detected at the beginning of the 2003/04 season and the broad diversity of strains co-circulating at that time. As the season progressed, the increase in incidence of symptomatic infections seen with GII-4 may have been associated with the loss of short-term immunological protection. The lack of diversity and the predominance of GII-4 strains throughout the 2004/05 and at the beginning of the 2005/06 season occurred during a period of lower-than-normal activity and may represent a diminishing number of individuals susceptible to symptomatic infection. This also suggests a better fitness for GII-4 strains as the many NoV genotypes co-circulating at this time remained endemic and a predominant genotype did not emerge as an epidemic strain. Herd immunity to GII-4 strains may have reached significant levels by the middle of the 2005/06 season and allowed an increase in the diversity of co-circulating NoV genotypes seen at this time. Also, this may represent an opportunity, through immunological pressure, for the selection and rapid spread of a new variant strain of the GII-4 genotype in a similar way to influenza virus populations, from which novel variants, characterised by genetic drift, are selected and become predominant due to the immunologically naïve pool of human hosts [22].

Detailed genetic analysis of GII-4 strains circulating from September 2003 to June 2006 revealed the presence of five variants of the GII-4 genotype with GII-4 v2, v3, v4, v5 and v6, being associated with a significant number of outbreaks of gastroenteritis. By June 2006, GII-4 v4 was the predominant strain associated with outbreaks of gastroenteritis in the UK, and it selection may have prolonged the normal NoV season.

Detailed genetic analysis of co-circulating NoV strains may provide insights into the natural history of NoV infections. It is clear that GII-4 strains dominate the NoV population infecting humans and clearly have an, as yet unidentified, advantage over other co-circulating genotypes. This may be a replicative advantage, a greater transmissibility associated with a lower infectious dose, a larger proportion of the population susceptible through inherited genetic factors, better survival of the virus in the environment, or a mechanism that allows the virus to evade immune surveillance to some degree.

Clearly, part of the success of this genus of the family *Caliciviridae* is associated with the relatively short-term protective immunity conferred by infection. The observations presented here are compatible with appreciable ''herd'' immunity resulting from a major epidemic associated with novel antigenic variants not seen previously in the human population. This could result in their ability to spread rapidly in an immunologically naive population. Interestingly, the introduction of a new strain is often associated with higher attack rates and an unusual pattern with infection occurring out of season [23]. Analysis of the data presented here and that detailing previous GII-4 variants [24] suggest that a new variant with increased fitness may be selected every 2–3 years from a pool of co-circulating virus variants. Similarly, in studies of the evolutionary changes seen with influenza A virus, the general pattern of evolutionary drift is characterised by non-random clusters of highly related genotypes that replace each other every 2–5 years [22].

Although there are parallels between the evolution of NoVs and influenza viruses, there are also significant differences. With NoVs, it is unlikely that the population susceptible to infection is reduced by infection and the resulting development of protective immunity. Rather, as immunity is short-lived and unlikely to be sterilising, the population oscillates between susceptibility to infection and susceptibility to infection with disease. Although GII-4 strains predominate, they do so within a population of co-circulating genotypes, and predominant strains replaced by novel variants do not become extinct but continue to circulate at lower levels within the human population.

Although genetic diversity among NoVs is associated with both recombination and the accumulation of point mutations through the error-prone nature of viral RNA replication, it is becoming apparent that the accumulation of point mutations, probably at sites encoding antigenic regions, results in virus variants that have a greater impact on public health. Recombination between two co-circulating

human NoV strains is unlikely to have such an impact as that seen through reassortment between animal and human influenza viruses, as unlike influenza viruses, the NoV recombinants identified to date bring nothing new to the pool of antigens currently circulating in the population.

Studies of virus variants, both fit and less fit, may provide insights into the mechanisms involved in virus fitness and immune evasion. Characterisation of regions of the genome encoding antigenic structures needs to be performed in order to determine if variants, defined through the identification of conserved nt or aa changes in the shell (S) domain are reproduced in, and significantly change, the antigenic properties of the protruding (P) domain. It needs to be determined whether genotypic changes translate to significant phenotypic changes that have an impact on public health.

References

1. Adak GK, Meakins SM, Yip H, Lopman BA, O'Brien SJ (2005) Disease risks from foods, England and Wales, 1996–2000. Emerg Infect Dis 11: 365–372
2. Domingo E, Diez J, Martinez MA, Hernandez J, Holguin A, Borrego B, Mateu MG (1993) New observations on antigenic diversification of RNA viruses: antigenic variation is not dependent on immune system. J Gen Virol 74: 2039–2045
3. Evans MR, Meldrum R, Lane W, Gardner D, Ribeiro CD, Gallimore CI, Westmoreland D (2002) An outbreak of viral gastroenteritis following environmental contamination at a concert hall. Epidemiol Infect 129: 355–360
4. Gallimore CI, Cubitt D, du Plessis N, Gray JJ (2004) Asymptomatic and symptomatic excretion of noroviruses during a hospital outbreak of gastroenteritis. J Clin Microbiol 42: 2271–2274
5. Gallimore CI, Cubitt D, Richards A, Gray JJ (2004) Diversity of enteric viruses detected in patients with gastroenteritis in a tertiary referral paediatric hospital. J Med Virol 73: 443–449
6. Gallimore CI, Cheesbrough JS, Lamden K, Bingham C, Gray JJ (2005) Multiple norovirus genotypes characterised from an oyster-associated outbreak of gastroenteritis. Int J Food Microbiol 103: 323–330
7. Gallimore CI, Pipkin C, Shrimpton H, Green AD, Pickford Y, McCartney C, Sutherland G, Brown DWG, Gray JJ (2005) Detection of multiple enteric viruses within a foodborne outbreak of gastroenteritis: an indication of the source of contamination. Epidemiol Infect 134: 41–47
8. Gallimore CI, Taylor C, Gennery AR, Cant AJ, Galloway A, Iturriza-Gomara M, Gray JJ (2006) Environmental monitoring for gastroenteric viruses in a primary immunodeficiency unit. J Clin Microbiol 44: 395–399
9. Gray JJ, Cunliff C, Ball J, Graham DY, Desselberger U, Estes MK (1994) Detection of immunoglobulin M IgM, IgA, and IgG Norwalk virus-specific antibodies by indirect enzyme-linked immunosorbant assay with baculovirus-expressed Norwalk virus capsid antigen in adult volunteers challenged with Norwalk virus. J Clin Microbiol 32: 3059–3063
10. Gray JJ, Green J, Cunliffe C, Gallimore C, Lee JV, Neal K, Brown DWG (1997) Mixed genogroup SRSV infections among a party of canoeists exposed to contaminated recreational water. J Med Virol 52: 425–429
11. Green J, Wright PA, Gallimore CI, Mitchell O, Morgan-Capner P, Brown DWG (1998) The role of environmental contamination with small round structured viruses in a hospital outbreak investigated by reverse-transcriptase polymerase chain reaction assay. J Hosp Infect 39: 39–45
12. Green KY, Ando T, Balayan MS, Berke T, Clarke IN, Estes MK, Matson DO, Nakata S, Neill JD, Studdert MJ, Thiel HJ (2000) Taxonomy of the Caliciviruses. J Infect Dis 181: S322–S330
13. Ho M, Monroe SS, Stine S, Cubitt D, Glass RI, Madore HP, Pinsky PF, Ashley C, Caul EO (1989) Viral gastroenteritis aboard a cruise ship. Lancet 2: 961–965
14. Jiang X, Turf E, Hu E, Barrett E, Dai XM, Monroe S, Humphrey C, Pickering LK, Matson DO (1996) Outbreaks of gastroenteritis in elderly nursing homes and retirement facilities associated with human caliciviruses. J Med Virol 50: 335–341
15. Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, Takeda N, Katayama K (2003) Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J Clin Microbiol 41: 1548–1557
16. Kaplan JE, Gary GW, Baron RC, Singh N, Schonberger LB, Feldman R, Greenberg HB (1982) Epidemiology of Norwalk gastroenteritis and the role of Norwalk virus in outbreaks of acute nonbacterial gastroenteritis. Ann Intern Med 96: 756–761
17. Kaplan JE, Schonberger LB, Varano G, Jackman N, Bied J, Gary GW (1982) An outbreak of acute nonbacterial gastroenteritis in a nursing home. Demonstration of person-to-person transmission by temporal clustering of cases. Am J Epidemiol 116: 940–948
18. Kobayashi S, Morishita T, Yamashita T, Sakae K, Nishio O, Miyake T, Ishihara Y, Isomura S (1991) A large outbreak of gastroenteritis associated with a small round structured virus among schoolchildren and teachers in Japan. Epidemiol Infect 107: 81–86
19. Kojima S, Kageyama T, Fukushi S, Hoshino FB, Shinohara M, Uchida K, Natori K, Takeda N, Katayama K

(2002) Genogroup-specific PCR primers for detection of Norwalk-like viruses. J Virol Methods 100: 107–114
20. Kuritsky JN, Osterholm MT, Greenberg HB, Korlath JA, Godes JR, Hedberg CW, Forfang JC, Kapikian AZ, McCullough JC, White KE (1984) Norwalk gastroenteritis: a community outbreak associated with bakery product consumption. Ann Intern Med 100: 519–521
21. Lees D (2000) Viruses and bivalve shellfish. J Food Microbiol 59: 81–116
22. Levin SA, Dushoff J, Plotkin JB (2004) Evolution and persistence of influenza A and other diseases. Math Biosci 188: 17–28
23. Lopman BA, Reacher M, Gallimore C, Adak GK, Gray JJ, Brown DW (2003) A summer time peak of ''winter vomiting disease'': Surveillance of Noroviruses in England and Wales, 1995 to 2002. BMC Pub Health 3: 1–4
24. Lopman B, Vennema H, Kohli E, Pothier P, Sanchez A, Negredo A, Buesa J, Schreier E, Reacher M, Brown D, Gray J, Iturriza M, Gallimore C, Bottiger B, Hedlund KO, Torven M, von Bonsdorff CH, Maunula L, Poljsak-Prijatelj M, Zimsek J, Reuter G, Szucs G, Melegh B, Svennson L, van Duijnhoven Y, Koopmans M (2004) Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. Lancet 363: 682–688
25. Marks PJ, Vipond IB, Carlisle D, Deakin D, Fey RE, Caul EO (2000) Evidence for airborne transmission of Norwalk-like virus (NLV) in a hotel restaurant. Epidemiol Infect 124: 481–487
26. Parashar UD, Dow L, Fankhauser RL, Humphrey CD, Miller J, Ando T, Williams KS, Eddy CR, Noel JS, Ingram T, Bresee JS, Monroe SS, Glass RI (1998) An outbreak of viral gastroenteritis associated with consumption of sandwiches: implications for the control of transmission by food handlers. Epidemiol Infect 121: 615–621
27. Smith DB, McAllister J, Casino C, Simmonds P (1997) Virus 'quasispecies' making a mountain out of a molehill. J Gen Virol 78: 1511–1519