High Prevalence of Prolonged Norovirus Shedding and Illness among Hospitalized Patients: A Model for In Vivo Molecular Evolution

J. Joukje Siebenga,1,2 Mathias F. C. Beersma,2 Harry Vennema,1 Paula van Biezen,3 Nico J. Hartwig,4 and Marion Koopmans1,2

1National Institute for Public Health and the Environment, Bilthoven, and Departments of 2Virology, 3Internal Medicine, and 4Pediatrics, Erasmus Medical Center, Rotterdam, the Netherlands

During a 2-year survey in an academic hospital, 8 (8.4%) of all norovirus (NoV)–positive patients showed prolonged norovirus illness and shedding (duration, 21–182 days). All patients had underlying illnesses, resulting in some level of immunodeficiency in 5. Four patients were admitted to the hospital with gastroenteritis, 2 acquired norovirus while hospitalized, and 2 were outpatients. Genotypes GII.4 and GIIb-GII.3 were found. Reinfection occurred in 3 patients. Full capsid sequences were determined from strains detected in sequentially collected stool specimens to study evolution. The greatest number of amino acid mutations in a given patient was 11; they were detected in NoV isolates recovered over a 119-day period and were mapped to positions at or near putative antigenic sites. In the patient with most severe immune dysfunction, only 5 amino acids mutated over 182 days, suggesting immune-driven selection. The severe impact on patients and hospitals and the potential role of prolonged shedders as a reservoir for viral antigenic variants lead us to stress the importance of confinement of outbreaks of NoV infection that occur in hospitals.

Noroviruses (NoVs) are a genetically and antigenically diverse genus of the Caliciviridae family and are the leading cause of acute gastroenteritis in people of all ages [1–4]. Episodes last 12–60 h and mainly entail vomiting and diarrhea. Although NoV illness is usually self-limiting, the burden of disease is considerable, with many people affected and a potential great impact in health–care settings [5–7]. Between 1994 and 2005, a total of 74% of all reported outbreaks of viral gastroenteritis in the Netherlands were caused by NoV, and up to 68% of these were caused by GII.4 strains [1, 8]. Successive GII.4 variants caused global epidemics, having accumulated mutations in a stepwise manner [8–13]. As we described elsewhere [12], the GII.4 variants are thought to result from an immune-driven selection process known as epochal evolution. It is unclear where these variants arise. It was previously suggested in a Swedish study that they may evolve in chronically infected patients [14]. This study described a case in which, over the course of 1 year, 11 amino acid mutations accumulated in the capsid protein of a GII.3 strain (ARG320/1999/US-like) that was infecting an immunocompromised patient. The number of amino acid mutations reported by Nilsson et al. [14] is similar the number that distinguish variants from each other (range, 8–25 mutations), and the mutations approximately mapped to the region where most variant-distinguishing amino acids are located [12, 14]. Only 2 other studies of prolonged NoV shedding have been reported. One describes a child with T cell deficiency who was infected with a GII.3 strain (also ARG320/1999/US-like) [15]. In virus recovered from this patient over a 6-month period, no amino acid mutations were found in a partial capsid sequence of 277 nucleotides in the 5' part of ORF2. A recent article described shedding of NoV for 6–7 weeks in 3 of 71 children who presented to
a pediatric clinic because of acute gastroenteritis; these children recovered clinically within a short period [16]. This study presented no information on NoV genotypes or other genetic data. Little more is known about the prevalence of chronic NoV infection.

This study was conducted as part of a larger survey of the role of NoV infections in a tertiary care hospital (unpublished data). We selected patients hospitalized during 2005–2006 who tested positive for NoV for at least 3 weeks. We identified prolonged shedding and illness in 11 (8.4%) of all NoV-positive patients. GIL4 and GIIb–GII3 recombinant strains were found. We determined changes in the gene encoding the NoV capsid during the course of illness for 8 patients. The rate of mutation accumulation was lowest among NoV recovered from patients who had the most severe immune impairment.

PATIENTS, MATERIALS, AND METHODS

Study population. Patients were retrospectively selected during a review of laboratory records from 2005–2006 at Erasmus Medical Center, an 1100-bed tertiary-care hospital with ~30,000 patients annually. NoV diagnostic tests had been requested for 899 samples from 502 patients admitted during 2005 and for 1084 samples from 571 patients admitted during 2006. Routine polymerase chain reaction (PCR) analysis was used for detection of genogroup I and II NoVs; 38 patients in 2005 and 93 in 2006 had positive PCR results. Patients were included in this study if fecal samples recovered during a period of ≥3 weeks tested positive for NoV and if ≥2 fecal samples were available. To compare the mutation-accumulation rates among NoV from patients with underlying illnesses with those among NoV from otherwise healthy patients, we analyzed a series of specimens from a female child (age, 4 months) who had a 2-day episode of NoV infection but no underlying illness.

RNA isolation and sequencing. We investigated stool specimens that were originally collected for diagnostic analysis, which included testing for adenovirus, rotavirus, astrovirus, norovirus, and enterovirus. Only a single patient (patient 4) tested positive for adenovirus, which was detected in a single sample. Unused portions of the specimens were subsequently stored at -80°C.

Fresh RNA was extracted from stool specimens in accordance with the methods of Svraka et al. [1]. Sequencing was done as previously described [12, 17, 18]. The MagNAPureLC total nucleic acid isolation kit (Roche Diagnostics GmbH) was used for extraction, in accordance with the recommendations of the manufacturer. Overlapping fragments of viral RNA were then reverse transcribed using AMV-RT (Invitrogen), yielding cDNA that was amplified and subsequently sequenced using the ABI-PrismBigDye Terminator v3.0 Ready Reaction Cycle kit. The same primers were used for amplification and sequencing.

Data processing. DNA sequences were processed, aligned, and analyzed using BioNumerics software (Applied Maths BVBA [Sint-Martens-Latem]). Additional analyses used BioEdit Sequence Alignment Editor 7.0.1 (Isis Pharmaceuticals) and DNASP 4.10.

Nucleotide sequence accession numbers. Capsid sequences of the first and last NoV isolate recovered from each patient were submitted to the DNA Database of Japan and assigned accession numbers AB385626 through AB385643.

RESULTS

Characteristics of patients. For 11 (8.4%) of all NoV-positive patients, stool samples recovered over a period of ≥3 weeks tested positive for NoV. Three patients were excluded from detailed analyses because samples were no longer available or because samples failed to yield positive PCR results, leaving 8 patients for the study. Samples were obtained from 2 patients in 2005, and samples from 9 patients were collected in 2006. Demographic and clinical characteristics of the 8 study patients are shown in figure 1 and table 1. Five patients were aged <3 years, and 3 were aged 37, 67, and 69 years. All patients had underlying conditions of varying nature. Patients 1–4 and 7 were immunocompromised, of whom 3 patients had leukopenia, and 1 had severe lymphocyte dysfunction after allogenic stem cell transplantation (table 1). The 3 nonimmunocompromised patients did not receive immunosuppressive medication.

Characteristics of stool samples and gastroenteritis. Samples were originally obtained from the patients for diagnostic purposes. Figure 1 shows the times of hospitalization and sampling and the course of gastroenteritis. Patients 2 and 4–6 were admitted to the hospital with gastroenteritis symptoms, patients 1 and 8 first showed gastroenteritis symptoms 6 and 4 days after admission, and patients 3 and 7 were outpatients (table 1).

The shortest sample series involved patient 3, for whom 2 samples remained available for the current analysis. Five samples had originally been recovered over 32 days from this patient, and all tested positive for NoV. Three specimens each were available from patients 4 and 6. Specimens were missing for both patients, and gastroenteritis symptoms lasted longer than the interval marked by the 3 available samples. One sample from patient 4 tested positive for adenovirus; this patient died as a result of poor health complicated by gastroenteritis. Patient 2 had the longest series, consisting of 11 samples recovered over 182 days. Patient 2 had severe gastroenteritis throughout this period and died possibly as a result of NoV infection. Patient 8 was coinfected with parechovirus (data not shown) and had no symptoms after 2 weeks. Gastroenteritis complaints reappeared after 4 weeks, after which the patient had diarrhea and vomiting for a number of weeks; hospital records indicate “an increase of symptoms, initially with vomiting” 65 days after the first sample was taken.
Collection of follow-up samples from the patients after the causative agent of gastroenteritis complaints had been established was generally poor, especially after hospital discharge. The exception was patient 7, who did not report gastroenteritis symptoms but was still PCR positive while the current study was being performed. At our request, 2 additional samples were obtained from him during outpatient clinic visits, lengthening the series duration from 56 to 119 days.

No spatial or temporal relation could be identified among the gastroenteritis episodes in the patients described here. Those who were admitted to the same ward were hospitalized at different time points (at least 5 months apart) and had different viral strains.

Findings of sequence analyses. Patients 2–4, 6, and 7 were infected by strains typed as GII.4 throughout their infection. Patient 1 had a strain with polymerase-type GIIb during the course of infection, and patients 5 and 8 were initially infected by GIIb strains before being infected by GII.4 strains. For all GIIb strains, the capsid genotypes belonged to GII.3 (ARG320/1999/US-like). The observation that patient 5 had 2 separate episodes of NoV illness and shedding, caused by 2 different NoV strains, excludes this patient from our definition of prolonged shedding, leaving 7 patients who shed the same virus for >3 weeks and showed prolonged illness. Patient 8 showed prolonged illness during his GIIb episode, but this was not confirmed for the GII.4 episode.

GII.4 strains belonged to variants 2004 (recovered from patients 2 and 5), 2006a (recovered from patients 4, 6, 7 and 8), and 2006b (recovered from patient 3). Figure 2A and 2B show the neighbor-joining trees of the GII.4 and GII.3 strains. Reference strains of the GII.4 variants have been included. In sequential strains from all 8 patients, mutations were found in the capsid (figure 2). The sequence patterns for a number of nucleotides showed the presence of ambiguous sites before complete substitution (data not shown). Fixation of these mutations was seen in the 5 long series but could not be confirmed for the short series because of low sample numbers. The virus isolated from stool specimens from patient 4, of which only 3 were available, had no nucleotide mutations after 14 days but had 15, resulting in 8 amino acid changes in the capsid, after 33 days. No difference was found in the partial polymerase sequences used for genotyping the strain.

Figure 3 shows rates of mutation accumulation and trend lines for patients with the longest series (i.e., patients 1, 2, and 7). We also determined the mutation-fixation rate of NoV shed by a child who had gastroenteritis symptoms for 2 days but no underlying illnesses (data are not shown in detail; 9 samples that were PCR positive for GIIb-GII.3 recombinants were recovered over 34 days, with a total accumulation of 6 nucleotide and 4 amino acid mutations in the capsid). NoV from this otherwise healthy child had a fixation rate of 0.13 amino acids/day. Viruses from patients 1 and 7, with moderate cellular immune deficiency and normal levels of serum immunoglobulins, had a fixation rate of 0.07 amino acid mutations/day; NoV from patient 2 had a fixation rate of 0.03 amino acid mutations/day. It is interesting to note that patient 2 had severe humoral immunodeficiency and no detectable serum levels of IgA and IgM. When the first and last samples of each series were compared, the ratio of non-
<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Disease</th>
<th>Effect on immune system</th>
<th>Leukocyte level[^a]</th>
<th>Lymphocyte %[^b]</th>
<th>Immunosuppressive therapy</th>
<th>Series duration, d</th>
<th>Samples recovered, no.</th>
<th>NoV shedding duration, wk</th>
<th>Gastroenteritis</th>
<th>Symptoms</th>
<th>Onset (coinfecting pathogen)</th>
<th>Time of first sample collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>4 mo</td>
<td>Acute myeloid leukemia</td>
<td>Aplasia during chemotherapy</td>
<td>1.4 ± 0.21</td>
<td>51–94</td>
<td>Chemotherapy</td>
<td>88</td>
<td>7</td>
<td>13</td>
<td>Intermittent episodes of vomiting followed after 2 d by diarrhea</td>
<td>Intermittent episodes of vomiting followed after 2 d by diarrhea</td>
<td>6 d after HA</td>
<td>6 d after HA</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>67 y</td>
<td>Churg-Strauss syndrome</td>
<td>Immunoglobulin deficiency (lgA level, &lt;0.01 g/L; lgM level &lt;0.03 g/L)</td>
<td>NR</td>
<td>NR</td>
<td>Dexamethasone (1 mg 3 times/d), mycophenolate, mofetil (750 mg 2 times/d)</td>
<td>182</td>
<td>11</td>
<td>26</td>
<td>Diarrhea and no vomiting; patient died</td>
<td>Diarrhea and no vomiting; patient died</td>
<td>Before HA</td>
<td>3 d after HA</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>69 y</td>
<td>T cell non-Hodgkin lymphoma</td>
<td>Severe T cell depletion</td>
<td>2.3–5.0</td>
<td>3–5</td>
<td>Prednisone (50 mg 1 time/d), alemtuzumab</td>
<td>21</td>
<td>2</td>
<td>3</td>
<td>Diarrhea and no vomiting</td>
<td>Diarrhea and no vomiting</td>
<td>Before HA</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>29 mo</td>
<td>Wiskott-Aldrich syndrome, allogenic stem cell transplantation</td>
<td>Dysfunctional CD4+/CD8+ lymphocytes</td>
<td>6.1–10.5 (NR)</td>
<td>20–48 (NR)</td>
<td>None</td>
<td>33</td>
<td>3</td>
<td>5</td>
<td>Diarrhea and no vomiting; patient died</td>
<td>Diarrhea and no vomiting; patient died</td>
<td>Before HA (adenovirus)</td>
<td>3 d after HA</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>9.5 mo</td>
<td>Down syndrome, megacolon, congenital defects</td>
<td>None</td>
<td>NR</td>
<td>NR</td>
<td>None</td>
<td>51</td>
<td>5</td>
<td>1</td>
<td>Intermittent episodes of vomiting and diarrhea</td>
<td>Intermittent episodes of vomiting and diarrhea</td>
<td>Before HA</td>
<td>2 d after HA</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>3.5 mo</td>
<td>Dilated cardiomyopathy</td>
<td>None</td>
<td>NR</td>
<td>NR</td>
<td>None</td>
<td>33</td>
<td>3</td>
<td>5</td>
<td>Diarrhea and no vomiting</td>
<td>Diarrhea and no vomiting</td>
<td>Before HA</td>
<td>17 d after HA</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>37 y</td>
<td>Seminal testis tumor, idiopathic thrombocytopenic purpura</td>
<td>CD4+/CD8+ cell lymphopenia</td>
<td>4.2 ± 2.7</td>
<td>9–17</td>
<td>Prednisone (40 mg 1 time/d)</td>
<td>119</td>
<td>8</td>
<td>17</td>
<td>Vomiting for 2 d, followed by diarrhea, that cleared after 2 wk</td>
<td>Vomiting for 2 d, followed by diarrhea, that cleared after 2 wk</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>3 mo</td>
<td>Very-long-chain acyl-CoA dehydrogenase deficiency, cardiomyopathy</td>
<td>None</td>
<td>NR</td>
<td>NR</td>
<td>None</td>
<td>65</td>
<td>4</td>
<td>4</td>
<td>Intermittent episodes of vomiting and diarrhea, cleared and reappearing, intensified after 65 d</td>
<td>Intermittent episodes of vomiting and diarrhea, cleared and reappearing, intensified after 65 d</td>
<td>After HA (parechovirus)</td>
<td>4 d after HA</td>
</tr>
</tbody>
</table>

**NOTE.** d, days; HA, hospital admission; mo, months; NA, not applicable; NR, normal range; wk, weeks; y, years.

[^a]: Data are mean ± SD or range 10^9 cells/L. NR, 3.5–10^9 cells/L.

[^b]: Data are percentage of the total number of white blood cells. NR, 15%–50%.

[^c]: During the period recorded in the medical records.
synonymous mutations in NoV from these samples was sometimes far greater than 1 (data not shown).

In the GII.4 strains, 15 of 34 nonsynonymous nucleotide mutations were located in the P1 domain, affecting 13 amino acids (244, 246, 248, 258, and 268 in the N-terminus and 407, 409, 411, 413, 479, 486, 497, and 519 in the C-terminus); 14 were located in P2, affecting 9 amino acids (295, 317, 327, 340, 375, 378, 389, 393, and 397); 1 was in the N domain (amino acid 33); and 4 were in the S domain (amino acids 93, 98, 130, and 174). Amino acids 174, 244, 340, 378, 389, 393, 397, 407, 413, and 497 were previously identified as informative sites in GII.4-variant transitions, and amino acids 340 and 407 changed with every variant transition [12]. Only in the P1 and P2 domains were identical amino acid mutations observed in different patients. Patients 4 and 7 both had amino acid substitutions at positions 248 and 258. In patients 2 and 6, G295 mutated to N295. In patients 2 and 6, G295 mutated to N295. In patients 2 and 6, G295 mutated to N295. In patients 2 and 6, G295 mutated to N295.

Figure 2. A and B. Neighbor-joining trees of complete amino acid sequences, obtained by means of the Jukes and Cantor correction, for GII.4 (A) and GII.3 (B) norovirus strains. Bootstrap values are specified as a percentage of 1000 iterations.

Figure 3. Rate of accumulation of amino acid (aa) and nucleotide (nt) mutations among norovirus isolates recovered from patients with the 3 longest sample series and 1 otherwise healthy child. Day 0 corresponds to the first day of sampling. Trend lines were added for the aa mutation-accumulation rates.
and 5, R340 mutated; in patients 2 and 5, it mutated to G340 in the last sample, turning RRD into RGD. In patient 3, E340 changed into G340, turning KED into KGD. Similar to the RGD motif, KGD involved a cell-recognition and -binding motif [19].

Of the GII.3 strains, fewer samples were available and fewer mutations were seen, compared with the GII.4 strains. Strikingly, 5 of 8 mutations occurred in the C terminal part of the P1 domain, and only 2 mutations were located in the P2 domain.

Structural implications of amino acid polymorphisms. Figure 4 shows the affected amino acids in the previously modeled GII.4 capsid for each patient separately [12]. The amino acid that changed in 3 patients (340), establishing the presence of an extra RGD or RGD-like motif, is positioned in a loop on top of the P2 domain.

A number of mutating amino acids clustered on the surface of the protein. Amino acids 317, 327, 407, 409, 411, and 413 were located at the side of the protein of intradimeric interaction in an upward-oriented fold of the P1 domain next to the P2 domain. The residues in 1 protein were in steric proximity to the residues in the neighboring protein of another dimer. Residues 295, 375, and 378 were close together in loops on top of P2, as were 393 and 397 with 389. Amino acids 244, 246, and 248 were all in the same β-sheet in P1. However, amino acids 93, 98, 268, 497, and 519 were located inside the protein at various locations, and 98, 497, and 519 were situated in β-sheets. The substitutions that took place here were not drastic: A→S, S→G, T→I, V→I, and S→A.

DISCUSSION

We showed that significantly prolonged periods of gastrointestinal illness due to NoV infection, combined with shedding over unusually long durations, are more common than previously recognized. Eleven patients (8.4% of the total number of NoV-positive patients in the hospital) were identified in a 2-year period at an 1100-bed hospital. For 8, a sufficient quantity of sample material was available for analysis in the current study. Of the 11 patients initially included, 2 had samples obtained in 2005, and 9 had samples obtained in 2006. From 2006 onward, infection-prevention protocols were intensified, which led to collection of follow-up samples from immunocompromised patients after the first diagnosis, resulting in the availability of more series. The greater number of patients during 2006 can also be explained by the high number of off-season outbreaks and the subsequent winter epidemic, caused by the emergence of 2 new variants of GII.4 in the spring of 2006 [12] and by an increase of GIIb NoV in the pediatric wards in 2006 (unpublished data).
All 7 patients identified in this study as prolonged shedders of NoV had underlying illnesses. Five patients (patients 1–4 and 7) had impaired immunity, and 5 had prolonged gastrointestinal illness (patient 7 did not have prolonged gastrointestinal illness, and the course of illness for patient 8 was not clear from the laboratory records). Symptoms of prolonged illness mainly entailed diarrhea; no persistent vomiting was recorded. Patient 8 had coinfection with parechovirus, and patient 4 tested positive for adenovirus once. No other common gastroenteritis viruses were found. Thus, we cannot exclude that some of the gastroenteritis symptoms were caused by factors other than NoV, such as medication or other infections.

Strains belonging to 2 different NoV genotypes were found in ensuing samples from 3 of the initial 11 patients. These patients were probably reinfected with new strains, but it cannot be ruled out that the mixed strains were present at the beginning of their infection. The GI.4–2006a strain in patient 4 showed no mutations in the first 2 weeks but showed 15 nucleotide mutations between days 14 and 33. Although the strain still had the characteristic genetic make-up of a 2006a variant, the occurrence of such a large number of mutations in such a short period probably reflects a new infection with a different strain. Thus, 4 of the initial 11 patients had reinfections with 2 distinct viral strains, all likely nosocomial.

Persistent infections with common respiratory viruses in immunocompromised patients have been described for parainfluenza 3, influenza, respiratory syncytial virus, human rhinovirus, and others [20–23]. The clinical impact of such viruses in this risk group might be severe and includes an increased risk of nosocomial. Additionally, we sought to address the possibility that chronic shedders were sources of new NoV variants. The accumulation and fixation of mutations in 5 patients indicates that, although NoV could not be cleared, an immune response imposed pressure on the virus, causing it to modify its capsid protein to evade immune recognition. The rate of mutation fixation in the viral capsids seemed linked to the level of impairment of both cellular and humoral immunity. NoV from patient 2, who had no humoral immunity and had cellular immunity that was weakened by medication, showed the lowest rate (0.03 amino acids/day). Patients 1 and 7 had mildly impaired immunity and had virus that accumulated 0.07 amino acid mutations/day, and NoV from the otherwise healthy child had a fixation rate of 0.13 amino acid mutations/day. Properly functioning immunity thus seemed to induce a higher mutation-fixation rate. If the data from the article by Nilsson et al. [14] that described the Swedish chronic shedder are plotted similarly (data not shown), 0.04 amino acid mutations accumulated daily, fitting with our data. This patient received immunosuppressive medication, resulting in low total lymphocyte counts, low CD4+ cell counts, but normal immunoglobulin concentrations [14]. Interestingly, patient 2, who had virus with the lowest fixation rate, had no detectable IgA (concentration, <0.01 g/L), which is normally excreted in the intestine. The role assumed for immune pressure in directing the mutational pattern in these viruses is confirmed by our observation of high ratios of nonsynonymous to synonymous mutations found among viruses from all patients.

In our previous study, informative sites were determined by comparing subsequent epidemic variants of GI.4 strains. Ten of the changing amino acids recognized in present study were informative sites, and 4 were direct neighbors of informative sites [12]. Of these, amino acids 340 and 407 changed with every variant transition. Amino acid 340 is part of a putative RGD motif that appears and disappears from the sequence. Although the biological relevance of these individual sites remains to be determined, it is striking that 340 changed in 3 patients and 407 changed in 1 patient investigated here. This strengthens the idea that these amino acids are involved in immune evasion, as was also suggested by Lindesmith et al. [27]. When comparing the GI.3 amino acid substitutions found in this study with the substitutions reported by Nilsson et al. [14], the following 3 matches were detected: 310, 404, and 415. This consistency suggests a role in immune evasion for these amino acids.

The NoV genotypes that were found—GIib-GII.3 recombinant and GIb-GII.4 (variants 2004, 2006a and 2006b)—were also found in 2005 and 2006 during population surveillance that is ongoing in the Netherlands (~2.8% and ~60%, respectively, of all outbreak-associated NoV). It is remarkable that, both in this study and in the previously described cases of chronic NoV infection, strains with GIb-GII.3 ARG320/1999/US-type capsids were found so predominantly. A large proportion of the patients were young; very young, at the onset of illness, 5 of 8 were <3 years of age. The 3 patients who were initially selected but later excluded from the study were <1 year of age. All patients with GIib-GII.3 strains were young infants. This discrepancy in genotype distribution between age groups might be explained by the prevalence of strains going around in pediatric wards in the hospital, where outbreaks of both GIb NoV infection and GIib-GII.3 NoV infection were detected. Alternatively, GIib strains might be better equipped to establish long-term infections or infections in young children. This illustrates our limited understanding of NoV epidemiology.

Although the available evidence provides no definite proof, it is conceivable that similar chronic shedders are the reservoir in which antigenic variants of NoV arise; GIb.4 variants accumulated 8–25 amino acid mutations in the capsid over periods of ≥2 years [12]. The numbers of mutations found here are similar
to that number. However, even though we identified a surprisingly high number of patients with prolonged shedding durations in this study, this finding bears no relation to the much higher frequency of infection in the population, where shedding of virus may last up to 3 or 4 weeks after clearance of symptoms. We have shown here that NoV in healthy individuals accumulates mutations more rapidly than in immunocompromised patients. Therefore, healthy persons may be of more importance than chronic shedders. In addition, although chronic shedders remain ill (with gastroenteritis symptoms) and PCR positive for NoV, the data do not enable us to conclude that the virus shed by the patients is infectious. Still, considering that a single specific amino acid mutation has been shown to be sufficient to confer resistance to immunity in murine norovirus [28] or to increase the virulence of West Nile virus [29], it is clear that the changes observed in this study require further study.

Because of the frequency at which these prolonged infections occur, the possibly severe impacts they have both on patients and hospital alike, and the possibility that these patients are reservoirs in which antigenic variants evolve, we cannot overestimate the importance of adequately containment of outbreaks of NoV infection in health-care settings.

References


8 • JID 2008:198 (1 October) • Siebenga et al.