Molecular Epidemiology of Norovirus Outbreaks in Norway during 2000 to 2005 and Reverse Comparison of Four Norovirus Real-Time Reverse Transcriptase PCR Assays

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During the period from January 2000 to August 2005 a total of 204 outbreaks of norovirus gastroenteritis were diagnosed at the Norwegian Institute of Public Health. A clear increase in the norovirus activity was seen in healthcare institutions during the winter seasons. Polymerase sequence analysis of norovirus strains from 122 outbreaks showed that 112 were caused by GII strains (91.8%). Two norovirus variants seen during the study period—GIib and GII.4—were predominant between January 2000 and September 2002, whereas GII.4 was predominant from September 2002 onward. The highest norovirus activity was seen during the 2002-2003 and 2004-2005 seasons with the emergence of new GII.4 variants. This study describes the molecular epidemiology of norovirus strains circulating in Norway during the five previous seasons and compares four norovirus real-time reverse transcriptase PCR assays. A suitable assay for routine diagnostics is suggested.

Noroviruses (NoVs) are estimated to be responsible for 60 to 80% of all human gastroenteritis outbreaks worldwide (4, 9, 16, 19, 23, 30, 34). Norovirus infections have become an increasing problem in healthcare institutions such as nursing homes and hospitals because outbreaks often result in closed wards (6, 15, 19, 22). Although the main mode of transmission in health institutions is person to person, NoVs are also efficiently transmitted via food, water, and contaminated surfaces (3, 17, 14, 21). A low infectious dose, short-lived immunity, and high stability in the environment make these viruses especially contagious (5, 7).

Noroviruses are classified into five genogroups (GI to GV), and human NoVs belong to genogroups GI, GII, and GIV (4, 17). Since no unified genotyping system for NoVs exists, genogroups can be divided into various numbers of genotypes (1, 13, 33, 37). The design of a single reverse transcriptase PCR (RT-PCR) assay for the detection of all genotypes has been a challenge due to the genomic diversity of NoVs (36). Earlier RT-PCR assays were based on the polymerase region since this was expected to be the most conserved. In a recent study, however, the highest degree of conservation was demonstrated in the polymerase-capsid junction region (12). Several broadly reactive real-time RT-PCR assays amplifying part of this junction have been developed (11, 12, 20, 26). The sensitivity has varied between the assays; however, an evaluation of the different assays using a common panel of strains has not been performed. Reliable and rapid NoV diagnostics is important for correct interventions in different settings, e.g., in institutions and in food-borne outbreaks.

Due to the predominance of assays amplifying the polymerase region, NoV genotyping has been mainly based on the polymerase typing system in epidemiological studies. Most of these studies describe a predominant circulation of strains belonging to GII, especially in health institutions (4, 15, 25, 27, 29, 32, 35). GII.4 has been the most common genotype in Europe during recent years, and two new genetic GI4L variants have emerged since January 2002 (19, 22, 25). Detailed genotype or sequence data are essential for the detection of new emerging variants and for the investigation of epidemiologically linked outbreaks.

The aims of the present study were to describe the molecular epidemiology of NoV strains circulating in Norway between 2000 and 2005, to compare four published NoV real-time RT-PCR assays on a panel of stool samples, and to use the best assay in the retesting of stool samples sent to the Norwegian Institute of Public Health between 1998 and 2004.

MATERIALS AND METHODS

Clinical specimens. (i) Molecular epidemiology. The Norwegian Institute of Public Health is the national reference laboratory for NoVs. Stool samples from outbreak cases of gastroenteritis were included in the study. The samples were received between January 2000 and August 2005. The majority of the samples were from outbreaks in hospitals and nursing homes in different areas of Norway. An outbreak of gastroenteritis was defined as two or more cases of acute gastroenteritis occurring in a given setting within a period of 2 weeks. No screening data on other enteric agents were available for these samples.

(ii) RT-PCR comparison. Four real-time RT-PCR assays (10, 11, 20, 31) were compared by using a panel of 97 stool samples analyzed in our laboratory between 1998 and 2004. The panel included 76 NoV-positive and 11 NoV-negative samples as classified by a polymerase RT-PCR published by Vinje et al. (34, 36). The remaining 10 samples had previously not been tested by the real-time RT-PCR. Based on the sequence data from the polymerase region of 42 NoV-positive samples, the following genotypes were included in the panel: G1L, G1L2, G1L3, G1L4, G1L5, G1L1, G1L2, G1L3, G1L4, G1L6, G1L7, G1L8, and G1L9.

Retesting of stool samples. A total of 372 stool samples randomly collected from selected periods between November 1998 and April 2004 were subsequently retested by the real-time RT-PCR assay that performed best on the...
panel. A total of 56 (15%) of these samples had previously tested positive and 316 (85%) had tested negative with the in-house diagnostic RT-PCR assay used at the time (the nested RT-PCR or the conventional one-step RT-PCR).

Sample preparation and RNA extraction. Stool extracts (10%) were prepared in phosphate-buffered saline (pH 7.2 to 7.4) by standard methods immediately after arrival at the laboratory (6). RNA was isolated from 140-μl unfrozen stool extracts by QiAmp microspin columns (viral RNA minikit; QIAGEN) according to the manufacturer’s protocol. The RNA was eluted in 60 μl of elution buffer and aliquots were stored at −70°C until testing.

Diagnostic RT-PCRs. Three different diagnostic RT-PCR assays were used during the study period. An in-house nested RT-PCR was used between January 1996 and November 2003 (32), a conventional one-step RT-PCR assay was used between November 2003 and December 2004 (34), and a real-time GI-GII duplex TaqMan assay has been used since January 2005 (11). All kits were used for amplification according to manufacturer’s protocol.

Real-time RT-PCRs included in the comparison. The primers and probes in the four published real-time RT-PCR assays (10, 11, 20, 31) are listed in Table 1. Assays A, B, and C were based on the TaqMan technology, whereas assay D was based on the SYBR green technology. The QuantiTect probe RT-PCR kit (QiAGEN) and the QuantiTect SYBR green RT-PCR Kit (QiAGEN) were used for amplification.

Some of the published reaction and cycling conditions were slightly modified to optimize the reactions and to adapt the performance to our real-time PCR machine. Optimization was performed on four fecal samples containing different NoVs (two GI and two GII). Assay A included primers (250 nM), GI probe (250 nM), and GII probe (400 nM), while assay C used 200 nM concentrations of each of the primers and probe (GII). The SYBR green assay D included 500 nM concentrations of each primer, and the reverse transcription conditions of each of the primers and probe (GII). The SYBR green assay D included 500 nM concentrations of each primer, and the reverse transcription conditions of each of the primers and probe (GII). The SYBR green assay D included 500 nM concentrations of each primer, and the reverse transcription conditions of each of the primers and probe (GII). The SYBR green assay D included 500 nM concentrations of each primer, and the reverse transcription conditions of each of the primers and probe (GII).
RESULTS

Outbreaks of NoV gastroenteritis in Norway from 2000 to 2005. During the five seasons studied, 204 of 310 gastroenteritis outbreaks were diagnosed as NoV positive in our laboratory. The majority of the outbreaks were from hospitals and nursing homes (171 of 204 [84%]), and the number of NoV outbreaks ranged between 9 and 67 per year (Table 2). Several other laboratories in Norway perform RT-PCR for NoVs, and the number of positive samples is routinely reported to our laboratory. These reports, however, do not allow us to determine whether the NoV-positive samples were from outbreaks or from sporadic cases. Figure 1 shows the monthly distribution of the NoV outbreaks diagnosed in our laboratory and the NoV-positive samples reported from all diagnostic laboratories. The positive samples diagnosed in our laboratory represent ca. 25% of all NoV-positive samples diagnosed in Norway. A clear increase was seen during winter and spring, with a peak between December and March. Between June and October, only a few NoV-positive outbreaks were observed. A total of 29 food- or waterborne (FW) outbreaks were detected during the study period, and all but one of these outbreaks were non-institutional. The usual seasonality was not observed with the FW outbreaks since 11 of 29 occurred between May and September. A marked increase in NoV activity was observed in September 2002, along with a change in the usual seasonal pattern and the emergence of the new GII.4-2002 variant. The new variant dominated the remainder of the extended 2002-2003 season. The NoV activity decreased during the next season, whereas an increase was again observed in November 2004 (Fig. 1). This rise in NoV activity in November 2004 concurrent with the emergence of a new GII.4 variant was similar to that observed in 2002.

Molecular epidemiology of NoVs. A total of 122 of 204 NoV outbreaks were randomly selected for sequencing analysis. Eight of the sequenced outbreaks occurred in 2000, 12 occurred in 2001, 29 occurred in 2002, 30 occurred in 2003, 24 occurred in 2004 and 19 occurred in 2005 (Table 2). A total of 112 of 122 outbreaks (91.8%) were caused by NoVs belonging to GII, and 94 of the GII isolates (84%) clustered within the GII.4 genotype. All but 2 of the 93 institutional outbreaks were caused by NoVs belonging to GII. Only 10 outbreaks were caused by GI strains, and 8 of these were associated with FW transmission. The remaining 17 sequenced FW outbreaks were caused by GII strains (17 of 29 [57%]). Seven GI outbreaks occurred between May and September. Polymerase sequence analysis revealed a variety of NoV genotypes circulating in Norway between January 2000 and August 2005 (Fig. 2): four GI genotypes (GI.1, GI.3b, GI.4, and GI.6) and seven GII genotypes (GII.1, GII.3c, GII.5, GII.2, GII.3, GII.4, and GII.7). Data for 10 GI outbreaks, the 25 sequenced FW outbreaks, the GII outbreaks not belonging to GII.4, and some of the panel

FIG. 1. NoV activity in Norway between January 2001 and August 2005. The figure shows the number of positive outbreaks (pos OB) analyzed in our laboratory and the total number of positive samples diagnosed in Norway. The food- or waterborne outbreaks are marked “FW OB.”

TABLE 2. Annual occurrence of the predominant NoV genotypes causing outbreaks of gastroenteritis in Norway between January 2000 and August 2005a

<table>
<thead>
<tr>
<th>Yr</th>
<th>NoV Sequenced</th>
<th>GII.4</th>
<th>GII.4-2002</th>
<th>GII.4-2004</th>
<th>GIIb</th>
<th>GI</th>
<th>FW</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>9</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2001</td>
<td>24</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>2002</td>
<td>67</td>
<td>29</td>
<td>26</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>2003</td>
<td>59</td>
<td>30</td>
<td>26</td>
<td>26</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2004</td>
<td>25</td>
<td>24</td>
<td>19</td>
<td>5</td>
<td>14</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>2005</td>
<td>20</td>
<td>19</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>204</td>
<td>122</td>
<td>94</td>
<td>53</td>
<td>30</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

a Results for food- or waterborne outbreaks (FW) are shown in the last column.
samples are presented Fig. 2. The remaining institutional GI.4 outbreaks not included in the phylogenetic tree are described in Table 2. The NoV strains detected during the 2000-2001 and 2001-2002 seasons grouped in two major clusters: one cluster being closely related to similar GI.1b strains detected in Spain and Sweden (2, 28) during the same period and the other cluster being closely related to the Farmington Hill strain. The nucleotide sequence homology between our GI.1b sequences and the GI.1b sequences from Sweden and Spain was between 97 and 99%. The nucleotide sequence homologies between our GI.4 sequences and the Farmington Hill strain was between 98 and 100% and between our GI.4 sequences and the Lordsdale prototype strain was ca. 90%. A complete dominance of the GI.4 strains was observed from September 2002 (Table 2). The nucleotide sequence homology between the GI.4 sequences detected since August 2004 and the new GI.4–2004 variant was between 98 and 100% (data not shown). The emergence of the new GI.4–2002 variant, with a shift in a specific motif from AACTTG to AATCTG (22), was registered for the first time in September 2002. This new variant dominated the two following seasons but was replaced by the other new GI.4–2004 variant in August 2004. The GI.4–2004 variant was detected in all but five outbreaks between August 2004 and August 2005. Apart from the GI.4 and GI.1b clusters, several outbreaks were caused by GI.2, GI.7, and GI.3b strains (Fig. 2).

Comparison of real-time RT-PCRs. Changes made to the reaction and cycling conditions yielded a higher analytical sensitivity (a lower cycle threshold \([C_T]\) value). Assays A, B, and D detected the same four GI genotypes included in the panel, while none of the assays detected the GI.2 genotype (Table 3). The panel contained 12 GI-positive samples, and 9 were detected by assay A, 10 were detected by assay B, and 11 were detected by assay D. The average \(C_T\) values for the nine GI samples detected by each assay were 25.7 for assay A, 30.7 for assay B, and 25.0 for assay D. The mean difference between \(C_T\) values in the two TaqMan assays A and B was 4.9 ± 2.6. The TaqMan assays A, B, and C showed identical results regarding the detection of the genotyped GII strains, whereas SYBR green assay D missed three of the samples. The average \(C_T\) values for the 25 GII-positive samples detected by all of the assays were 23.8 for assay A, 22.2 for assay B, and 22.6 for assays C and D. The mean difference between \(C_T\) values in the assays with highest and lowest average was 1.5 ± 1.2, which indicates that the assays have similar analytical sensitivity for detecting GI.1b strains included in the panel. The samples that tested negative in assays A and D were also negative in the monoplex versions of these two assays. The three TaqMan assays (A, B, and C) showed a high specificity for identifying correct NoV genogroup (Table 3). The melting-curve analysis in the assay D showed melting points in the range of 79 to 83°C. However, it was not possible to differentiate between GI and GII strains based on melting point (data not shown). In NoV-containing samples the melting-curve analysis showed no interference from unspecific polymerization. Gel visualization of six PCR products obtained in assay D verified the amplicon size and the absence of spurious products. Regarding the remaining 55 untyped stool samples, similar results were obtained with assays A, B, and C, whereas several samples were not detected by assay D. According to assays A and B, 4 of the 55 samples contained GI strains. These four strains were also detected by assay D (Table 3).

Based on the results from the conventional RT-PCR on 87 stool samples in the panel, 76 were NoV positive and 11 were NoV negative. Altogether, assay A detected 69 samples (13 GI and 56 GII), assay B detected 71 samples (14 GI and 57 GII), and assay D detected 61 samples. Assay C gave the highest score, with the detection of 58 GI-positive samples. The conventional RT-PCR detected the highest number of positive

### Table 3. Results from the comparison of four NoV real-time assays with a panel of 97 stool samples

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Assay A</th>
<th>Assay B</th>
<th>Assay C</th>
<th>Assay D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GI</td>
<td>GII</td>
<td>GI</td>
<td>GII</td>
</tr>
<tr>
<td>GI.1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>GI.2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GI.3</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>GI.4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>GI.5</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>9</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>GI.1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>GI.2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>GI.3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>GI.4</td>
<td>18</td>
<td>0</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>GI.6</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>GI.7</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>GIIb</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>GIIc</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>0</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Positive</td>
<td>34</td>
<td>4</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Negative</td>
<td>11</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Not tested</td>
<td>10</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

a A total of 87 samples had previously been tested by a conventional NoV RT-PCR and classified as NoV positive (n = 76) or negative (n = 11). Ten stool samples had not previously been tested. Genotyping of 42 strains was performed in the polymerase region. The table gives the number of samples with different GI and GII genotypes. All samples were tested by each of the assays.

b Assay A, duplex with TaqMan technology (11); assay B, monoplex (GI and GII) with TaqMan technology (10); assay C, monoplex (GII) with TaqMan technology (20); assay D, duplex with SYBR green technology (31).

c The positive samples include the four GI samples detected by assays A and B.

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**FIG. 2.** Phylogenetic tree derived from 12 NoV GI and 39 NoV GII strains detected in Norway during the study period. The phylogenetic tree was based on 240 bp from the polymerase region and was constructed by the neighbor-joining method using MEGA3 software. Branch lengths are related to the degree of divergence between the strains. The major branches are marked with GI and GII. Bootstrap values for branches of >50% are given. The food- or waterborne outbreaks are marked “FW,” and the institutional outbreaks are marked “INSTITUTION.” Data for GI outbreaks, FW outbreaks, GII outbreaks not belonging to GII.4, and some of the panel samples are presented. Two of the GI samples are only included in the stool panel.
samples (n = 76); however, four samples were only detected by the real-time assays.

Retesting of stool samples collected from 1999 to 2004. Of the 372 stool samples, 56 (15%) included in the retesting were previously classified as NoV positive based on the in-house diagnostic RT-PCR. In total, 96 samples (26%) tested as NoV positive when retested with assay A (91 GII and 5 GI). The 56 samples previously classified as NoV positive were also positive with assay A, whereas the 40 samples classified as NoV negative were all GII positive with assay A.

**DISCUSSION**

The clear increase in NoV activity seen in many European countries during autumn 2002 was due to the emergence of a new GII.4 variant. A similar trend was seen in Norway, as indicated by the significant increase in the number of positive samples analyzed in or reported to our laboratory. Figure 1 shows the variation in NoV activity observed during the five seasons studied. In accordance with other studies, the highest NoV activity was seen during the 2002-2003 season. Based on the number of positive samples reported to our institute, a high NoV activity was also recognized during the 2004-2005 season. This was similar to the high NoV activity observed in Finland, the Netherlands, and England during the same season (DIVINET). The increased NoV activity seen in Norway during these two seasons is most likely due to the emergence of the new GII.4 variants reported to the European Network in 2002 and 2004. Although the real number of NoV outbreaks occurring during the study period is unknown, the present study shows that the NoV activity and seasonality seen in Norway has been similar to that reported from other European countries.

The complete picture of different NoV genotypes circulating in Norway during the study period remains unknown. Nevertheless, the present study shows that there have been two dominant NoV clusters: GIIb and GII.4. Both of these genotypes were predominant during the 2000-2001 and 2001-2002 seasons, and the strains within the GIIb cluster showed 98 to 100% sequence homology to the GIIb strains from Spain, Sweden, and Finland in 2001 (2, 25, 28). The next season started with the emergence of the new GII.4-2002 variant, and the strains clustering with the GII.4 genotype have been predominant since 2002. This genotype has been shown to persist from year to year. A <5% nucleotide sequence difference was detected between the GII.4 sequences, and the different GII.4 variants clustered together in the phylogenetic tree (data not shown). Although the GII.4 genotype seems to undergo continuous genetic changes, only a few point mutations were seen in the polymerase region between the different GII.4 sequences. One of these mutations was detected in a specific motif, and the change in this motif reflected the GII.4 variant detected. However, to gain more insight into the pathogenic potential of the different variants, other regions, e.g., the capsid region, must be analyzed. The new GII.4-2002 variant appeared in Norway in September 2002, whereas the GII.4-2004 variant appeared in August 2004. The delayed detection of the new variants in Norway compared to other European countries was probably due to the limited number of outbreaks selected for sequencing. The new variants were detected in 83 of 94 GII.4 outbreaks between September 2002 and August 2005 (Table 2).

The GIIb and GII.4 genotypes have been associated with the majority of hospital outbreaks (4, 25), and this seems to be the case also in Norway. This was not surprising since the majority of outbreaks included in the present study were institutional. Due to the high hygienic level in hospitals, it has been speculated that only the most resistant NoV strains are capable of causing institutional outbreaks (25). This may be one explanation for the global distribution of GII.4 strains in hospital outbreaks. However, little is known about the environmental stability, the dose needed to produce an active infection, or the pathogenic potential of different NoV genotypes. Different strains may also induce different degrees of immunity. To our knowledge, different virulence factors have not yet been linked to different genotypes. Different strains may also induce different degrees of immunity. To our knowledge, different virulence factors have not yet been linked to different genotypes. The increased NoV activity seen in many European countries during autumn 2002 was due to the emergence of a new GII.4 variant. A similar trend was seen in Norway, as indicated by the significant increase in the number of positive samples analyzed in or reported to our laboratory. Figure 1 shows the variation in NoV activity observed during the five seasons studied. In accordance with other studies, the highest NoV activity was seen during the 2002-2003 season. Based on the number of positive samples reported to our institute, a high NoV activity was also recognized during the 2004-2005 season. This was similar to the high NoV activity observed in Finland, the Netherlands, and England during the same season (DIVINET). The increased NoV activity seen in Norway during these two seasons is most likely due to the emergence of the new GII.4 variants reported to the European Network in 2002 and 2004. Although the real number of NoV outbreaks occurring during the study period is unknown, the present study shows that the NoV activity and seasonality seen in Norway has been similar to that reported from other European countries.

The comparison of four published NoV real-time RT-PCR assays showed that assay A satisfied the combined requirements for sensitivity and simplicity. Real-time RT-PCR is less cumbersome and safer with regard to cross-contamination than conventional RT-PCR. To optimize our NoV diagnostics, a published conventional polymerase RT-PCR was compared to a SYBR green assay (assay D) amplifying a different part of the polymerase region and to three TaqMan assays amplifying the polymerase-capsid junction (assays A, B, and C). Assay A, which detects the most prevalent genotypes, was included in the comparison for the convenience of running a GI and GII duplex reaction. Johikumar et al. had previously demonstrated that assay A was more sensitive than the assay described by Kageyama et al. (11, 12). These two assays contain identical GII reverse primer and probe. Loisy et al. also demonstrated that the GI assay described by Kageyama et al. performed poorly on NoV-positive shellfish (20). Their modified version of the Kageyama GII primers and probe performed well and was therefore included in the present study (assay C). Assay B amplified a different part of the polymerase-capsid junction in GI strains compared to assay A, whereas the GII primers and probe were located in the same area as in assays A and C. SYBR green assay D had been shown to detect a broad spectrum of NoV genotypes (GI 1 to 5 and GII 1 to 8) and was included due to a different location in the polymerase region and the duplex form.
lished primers which performed well in an international study on NoV RT-PCR assays (36). The three TaqMan assays (A, B, and C) detected a similar number of NoV-positive samples. Although SYBR green assay D performed well on GI samples, the total number of positive samples detected was lower than with the TaqMan assays. These results are probably not due to a lower sensitivity of the SYBR green technology compared to the TaqMan technology. Some of the samples missed by the SYBR green assay D yielded low Ct values in the TaqMan assays. This indicates a high NoV copy number, and these samples should therefore be NoV positive even in a less sensitive assay. The observed Ct values for the assay D were also similar to the Ct values in assays A, B, and C. The assay D primers were located in a different part of the genome, and this may have resulted in a lower detection rate of GIHI strains (Table 3). The TaqMan assays showed equal NoV detection rates, and the main difference in performance between assays A and B is displayed in the Ct values from the genotyped GI samples. The mean difference in the Ct values was 4.9 ± 2.5, with assay A yielding the best result. This indicates that assay A has a higher analytical sensitivity than assay B, but this may only partly explain why Jothikumar et al. failed to detect the GI samples with assay B in their comparison of assays A and B (11). Regarding the clinical sensitivity, both assay A and assay B performed well in the present study. The lower analytical sensitivity observed in the detection of GI samples with assay B may be due to the location of primers and probes in a less conserved area of the polymerase-capsid junction (12). The conventional RT-PCR detected the highest number of positive samples. This may be due to a bias in the panel because it was classified by the same conventional RT-PCR. In addition, the real-time PCRs were run on RNA that may have been degraded during storage. On the other hand, the real-time assays detected NoV in four stool samples previously classified as NoV negative.

Although assays A and B showed equal clinical sensitivity in the present study, assay A showed a higher analytical sensitivity for GI strains compared to assay B. Therefore, assay A probably has an increased clinical sensitivity on fecal samples with a low viral load. The practical and economical advantages of a duplex assay were also considered important when assay A was established as the new NoV-diagnostic RT-PCR. Although assay D is a duplex assay and SYBR green is a less expensive technology, the lower detection rate for GIHI samples and the lack of differentiation between GI and GII strains make it less suitable as a diagnostic tool. Assay C gave a slightly higher detection rate compared to the other assays and could be used as a first-line GII screening method.

Retesting of 372 randomly chosen stool samples previously analyzed in our laboratory between 1998 and 2004 was performed with assay A. The goal was to achieve an extended comparison of assay A and the conventional RT-PCRs and to reveal whether false negatives had contributed to the low prevalence of NoV-positive samples in Norway. The number of positive samples increased from 56 (15%) to 96 (26%). The results demonstrate the increased clinical sensitivity of assay A compared to the former diagnostic RT-PCRs. However, the difficulty of designing an RT-PCR assay that detects all NoV strains is demonstrated by our results since assay A did not detect all of the positive stool samples in our panel. It is, however, difficult to estimate the true false-negative rate of the RT-PCR methods used in the present study. Electron microscopy could probably have detected some samples missed by RT-PCR, i.e., new genetic variants, but was not available in our laboratory.

In summary, the NoV activity observed in Norway has been similar to that seen in other European countries during the same period. The present study also indicates that the strains circulating in Norway are similar to the strains detected in other countries. In general, DIVINE-NET has demonstrated the importance of common sequence databases in the detection of new NoV variants. It is also important to further enhance the capacity for national and international surveillance across Europe of NoV outbreaks.

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REFERENCES


