Noroviruses (NoVs) are increasingly recognized as an important cause of nonbacterial gastroenteritis among individuals of all age groups worldwide (13). They are nonenveloped, single-stranded RNA viruses belonging to the family Caliciviridae. The 7.7- to 8.0-kb positive-sense single-stranded RNA genome encodes three large open reading frames (ORFs) (16). ORF 1 encodes nonstructural proteins, while the viral structural proteins are encoded by ORFs 2 and 3. NoVs are classified into five distinct genogroups (genogroup I [GI] to GV), of which genogroups I, II, and IV are known to cause human infections. Each genogroup is further subdivided into genotypes. Eight GI genotypes, 17 GII genotypes, and 1 GIV genotype have been identified so far (11, 22).

NoVs are increasingly recognized as the second most important cause of viral gastroenteritis in young children (11). A recent review of reports from both developed and developing countries indicates that the prevalence rates of NoV gastroenteritis in hospitalized children range from 6 to 48%, with the overall median being 14% (9). It has been estimated that NoVs may be responsible for more than 1 million hospitalizations and 200,000 deaths in children <5 years of age in developing countries (17).

There are recent reports of NoV outbreaks in neonatal nurseries associated with clinical presentations such as necrotizing enterocolitis (NEC), diarrhea, vomiting, abdominal distension, and fever (2, 18, 20). However, there are limited data on NoV infections in the neonatal population in nonoutbreak situations (19). The present study was conducted to determine the prevalence rates of GII NoVs among neonates with gastrointestinal (GI) disease and asymptomatic controls using a case-control design and to characterize the strains infecting neonates. This study focused on testing for GII NoVs, as previous hospital- and community-based epidemiological studies in southern India have demonstrated the predominance of the GII genotype and the limited circulation of GI viruses (12). The potential importance of these infections in neonates was highlighted by a recent notification on Promedmail (http://www.promedmail.org/archive. no. 2010521.1689), which reported six norovirus-associated deaths in neonates in Johannesburg, South Africa.

MATERIALS AND METHODS

Study design. The study was carried out at the Christian Medical College, a 2,234-bed tertiary-care hospital in Vellore, southern India, with 60 neonatal beds. A case-control study was carried out with a subset of samples collected for a study on the epidemiology and clinical manifestations of neonatal rotavirus infections (14), in which rotavirus was detected in stool samples using a commercial enzyme immunoassay for the detection of the VP6 antigen (Rotavirus IDEIA; Dako, Ely, United Kingdom). For this study, stool samples from neonates admitted to the nursery for more than 48 h with symptoms of diarrhea, vomiting, or NEC as well as equal numbers of asymptomatic controls from the same month, wherever available, were screened for NoV GII by reverse transcription-PCR (RT-PCR).

Clinical information. Demographic and clinical information was collected for all neonates enrolled in the study. Information regarding the gestational age, mode of delivery, reason for nursery admission, clinical findings, duration of hospitalization, and progress were collected.

RNA extraction and reverse transcription. Viral RNA was extracted from 20% (wt/vol) fecal suspensions in minimal essential medium (MEM) using the guanidium isothiocyanate/silica method described by Boom et al. (4). cDNA was generated by reverse transcription in the presence of random primers (hexamers) [Pd(N)6; Pharmacia Biotech, United Kingdom], using 400 units of Moleney murine leukemia virus reverse transcriptase (Invitrogen, Paisley, United Kingdom). The cDNA was stored at −20°C until further testing.

Detection and characterization of norovirus strains. The cDNA was used as the template for the detection of the RNA-dependent RNA polymerase gene (RdRp) of GII NoV using a nested RT-PCR. Published oligonucleotide primers
TABLE 1. Comparison of demographic characteristics between symptomatic and asymptomatic neonates

<table>
<thead>
<tr>
<th>Variable</th>
<th>Symptomatic (n = 161)</th>
<th>Asymptomatic (n = 148)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm</td>
<td>73 (48)</td>
<td>78 (52.7)</td>
<td>0.173</td>
</tr>
<tr>
<td>Term</td>
<td>87 (56)</td>
<td>68 (45.9)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>93 (53)</td>
<td>84 (56.8)</td>
<td>0.858</td>
</tr>
<tr>
<td>Female</td>
<td>68 (52)</td>
<td>64 (43.2)</td>
<td></td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower-segment cesarean</td>
<td>67 (41.6)</td>
<td>81 (54.7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Assisted</td>
<td>13 (8.1)</td>
<td>16 (10.8)</td>
<td></td>
</tr>
<tr>
<td>Normal vaginal</td>
<td>81 (50.3)</td>
<td>51 (34.5)</td>
<td></td>
</tr>
</tbody>
</table>

GR21 (5’ ACC ATT AAT GAG GGA CTA CC 3’) and GR22 (5’ GCT GTC AGT TTC TCT GGG TC 3’) were used to amplify a 203-bp fragment of the NoV GII RNA polymerase gene in the first-round PCR. Primers SR46 (5’ TGC AAT TCC ATC GCC CAC TGC TCC ACC 3’) and GR12 (5’ AGT TGT CAC GAT CTC ATC ACC 3’) were then used to amplify a 126-bp region nested within the GIIb RdRp gene. The sequences were resolved in an automated DNA sequencer (ABI Prism 310 genetic analyzer; Applied Biosystems), and electropherograms were analyzed using the BioEdit software package (version 7.0.5.3). Dendrograms were formed using the Neighbor-joining algorithm using 1,000 pseudoreplicates. Strains were inferred from the present study have been deposited in GenBank, and the accession numbers are GU120361 to GU120389.

RESULTS

Stool samples from 161 neonates with GI symptoms, including 70 cases with loose stools, 29 cases with vomiting, and 62 cases with NEC, and 148 asymptomatic controls were screened for GII NoV by nested RT-PCR. Analysis of demographic data showed no significant differences in the gestational ages or the male/female distributions between the two groups of neonates. However, significantly more neonates born by normal vaginal delivery than neonates born by lower-segment cesarean delivery or assisted delivery presented with gastrointestinal symptoms (Table 1).

NoV was detected in 81 (26.2%) of the 309 samples tested in the study. The rate of detection of NoV was significantly higher among the symptomatic neonates (60, 37.2%) than among the asymptomatic neonates (24, 14.1%) (χ² test, P < 0.001). However, analysis of individual clinical presentations showed no significant differences in the proportion of cases with loose stools or NEC among NoV-positive neonates in comparison with that among NoV-negative neonates. In contrast, a significantly higher proportion of NoV-negative neonates (19.8%) than NoV-positive neonates (5.7%) presented with vomiting (P < 0.001).

In this study, coinfection with rotavirus was seen in 55 samples, with 46 of the samples being from symptomatic neonates and 9 being from asymptomatic controls (P < 0.001). There were significantly more cases of loose stools among neonates with rotavirus-norovirus coinfection than among neonates with only rotavirus infection (P < 0.05), while there were significantly more cases of NEC among neonates with NoV infection than among those with a rotavirus-norovirus coinfection (P < 0.05) (Table 2). A significant association with vomiting was observed among rotavirus-norovirus-coinfected neonates compared with that among neonates with only norovirus infection (P < 0.05).

Strain characterization. Sequencing of NoV-positive PCR amplicons from the RdRp region was carried out for samples from 14 neonates with NEC and 15 asymptomatic controls. Twenty-seven sequences were identified as belonging to GIIb. Of these, the sequences in 26 samples were closest in identity to GIIb strain sequences (96 to 99%) described from children with diarrhea in western India, while the sequence of 1 sample showed 95% identity to that of a GIIb strain described from Japan. Two other strains, one from a symptomatic neonate and the other from an asymptomatic neonate, were identified as GII genotype 4 (GII.4) and had 96% identity to published sequences from Spain and France (5). To further characterize the virus identified, the capsid region of virus in a subset of six samples identified as GIIb in the RdRp region from both symptomatic and asymptomatic neonates was sequenced, and all viruses were found to belong to the GII.4 genotype (Fig. 1).

DISCUSSION

With increasing evidence for the role of NoV as an important gastrointestinal pathogen in children and recent reports of nosocomial outbreaks and mortality in neonatal nurseries, it is imperative to study the prevalence of NoV infections in neonates. In this study, NoV was detected in stool samples from 26.2% of neonates, and a significantly higher prevalence rate...
had loose stools. While the lack of association of NoV infection
while none of the neonates with an exclusive NoV infection
treated among neonates with norovirus-rotavirus coinfection,
viruses, and significantly more cases of loose stools were de-
one-third of symptomatic neonates had a coinfection with both
association of NoV with gastrointestinal symptoms. Nearly
determined if coinfection with rotavirus could contribute to the
important gastrointestinal pathogen in this setting (14), we

breakup of individual symptoms showed that the proportion of
of NoV infections with gastrointestinal symptoms was seen, a
vomiting, as well as recently described symptoms, such as nec-
tinal symptoms, we chose to study samples from infants pre-
colonitis. To determine the association of NoV with gastrointes-
ment was the predominant strain in this population. To the

was seen among symptomatic neonates, indicating an associa-
gastrointestinal disease. Sequencing of the RdRp region revealed that GIIb belonging to the GII.4 genotype was the predominant strain in this population. To the best of our knowledge, this is the first report characterizing NoV strains from symptomatic and asymptomatic neonatal infections, since the recent report from Johannesburg of neonatal deaths did not provide sequence-confirmed data.

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FIG. 1. Phylogenetic dendrogram constructed by the neighbor-

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Since rotavirus has previously been demonstrated to be an
important gastrointestinal pathogen in this setting (14), we
determined if coinfection with rotavirus may result in
significantly more cases of other types of gastrointestinal dis-
ease in this population.

A surprising finding from this study was the clonality of the
infected strains, with 93.1% of all RdRp regions sequenced
being identified as the RdRp region of the GIIb strain and all
capsid sequences belonging to the GII.4 genotype. These
strains showed a high degree of identity of the GIIb strains
from children with gastroenteritis in India (6). There are se-
veral recent reports of GIIb infection among children world-
wide, resulting in speculation that this strain may preferen-
tially cause infections in children (3, 10, 15). It is important to note
that no GIIb strains were described in previous concomitant com-

REFERENCES


Dutch tertiary care hospital (2002-2007): frequent nosocomial transmission