Occurrence of Norovirus Infections in Asymptomatic Food Handlers in South Korea

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Prevalence of asymptomatic norovirus infection was investigated in food handlers in South Korea. Among 6,441 subjects, 66 (1.02%) had norovirus infections confirmed by reverse transcription (RT)-PCR (real time and nested). GII-12 and GII-4 were the prevalent genotypes. Our data suggest that infection of asymptomatic food handlers is an important transmission source in norovirus outbreaks.

Norovirus (NoV) is considered the major cause of acute gastroenteritis in all age groups worldwide. NoV infections frequently result from person-to-person transmission in cruise ships, restaurants, hotels, nursing homes, schools, and hospitals. In addition, NoVs have been detected in environmental samples as well as in contaminated foods such as oysters, shellfish, sandwiches, salads, and even ice. Personal hygiene practices of infected food handlers are considered the most important contributing factor in the spread of food-borne diseases. Foods may be contaminated by contact with water that is contaminated by animal manure or human sewage or by unhygienic manipulation by a food handler excreting the virus, although this second cause is probably underestimated, because it is difficult to prove.

NoVs are plus-sense, single-stranded, nonenveloped RNA viruses belonging to the family Caliciviridae. The NoVs are classified into 5 genogroups (GI to GV) by phylogenetic analysis of the capsid protein. The GI and GII genogroups of human NoV are further classified into 14 and 21 genotypes, respectively.

Amplification of NoV RNA from fecal samples using primers targeting the capsid region of the viral genome and sequencing of the reverse transcription (RT)-PCR products are preferred for molecular characterization and typing of NoVs. Many studies report the monitoring of NoV in facilities with outbreaks. However, little information is available about circulating viral strains in asymptomatic individuals in facilities without NoV outbreaks. Recently, another epidemiologic study of NoV outbreak in South Korea has reported that the excretion of NoV from asymptomatic food handlers may be an infection source of NoV outbreak. We investigated the molecular epidemiology of NoV isolated from asymptomatic food handlers working at non-outbreak food catering facilities in South Korea from February 2009 to February 2010.

Fecal samples were collected from asymptomatic food handlers during regular physical examinations at 11 health centers in South Korea. Samples were diluted 1:10 in phosphate-buffered saline (pH 7.2), vortexed, and cleared by centrifugation (8,000 rpm, 10 min). Supernatants were stored at −70°C until use. For NoV detection, viral RNA was extracted using silica-coated magnetic beads combined with an automatic liquid handling system (Tecan, Switzerland), adapting Boom’s methods.

NoV was identified using real-time RT-PCR and conventional nested RT-PCR. Briefly, a 50-μl reaction mixture contained 5 μl of viral RNA, 1 μl (5 U) of reverse transcriptase, 25 μl of TaqMan universal RT-PCR master mix (Applied Biosystems), a 400 nM concentration of each primer, 10 pmol of RING1(a), and either 5 pmol of RING1(b) probe for NoV GI detection or 5 pmol of RING2 probe for NoV GII. Amplification was performed with

<table>
<thead>
<tr>
<th>Season</th>
<th>No. of samples (%)</th>
<th>No. of strains with genotypes:</th>
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<tbody>
<tr>
<td></td>
<td>NoV positive by:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Real-time PCR</td>
<td>Nested PCR</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 (0.16)</td>
<td>3 (0.08)</td>
</tr>
<tr>
<td>March to October 2009</td>
<td>3,718</td>
<td>3</td>
</tr>
<tr>
<td>(nonwinter)</td>
<td>60 (2.00)</td>
<td>33 (1.21)</td>
</tr>
<tr>
<td>November 2009 to February 2010 (winter)</td>
<td>2,723</td>
<td>31* 1 1 3 10 2 1 15 1</td>
</tr>
<tr>
<td>Total</td>
<td>6,441</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>66 (1.02)</td>
<td>36 (0.56)</td>
</tr>
</tbody>
</table>

* The NoV sequence could not be determined for 2 specimens.
an ABI Prism 7900 sequence detector (Applied Biosystems). Amplification data were collected and analyzed with Sequence Detector software version 2.1 (Applied Biosystems, Foster City, CA). In each experiment, an NoV GI- or GII-specific standard curve was generated by a 10-fold serial dilution (10^7 to 10^1 copies) of purified NoV GI or GII RNA standards synthesized by in vitro transcription. NoV genotype was determined by RT-PCR and seminested PCR as previously described (15). All PCR products were sequenced using an ABI Prism 3100 automatic sequencer and BigDye Terminator cycle sequencing mix (Invitrogen, Carlsbad, CA). Partial nucleotide sequences of capsid genes were aligned with ClustalW and compared to those of reference strains obtained from GenBank. Phylogenetic analysis was conducted with the MEGA program (version 4.0) using the neighbor-joining method, and statistical confidence for the evolutionary trees was assessed by bootstrap analysis (1,000 replicates) (16).

Among 6,441 food handlers, asymptomatic infection was identified in 66 (1.02%) by real-time RT-PCR (Table 1). The detection rate of asymptomatic infection in winter and nonwinter was 2.20% and 0.16%, respectively. In summer, 6 (0.16%) of 3,718 food handlers were asymptomatically infected by NoVs. Three of 6 GII-positivesamples were genotyped as GII-12 and GII-14, and the remaining 3 were not determined by sequence analysis (Table 1). Mean viral load of NoV GII in stool specimens was 6.3 × 10^4 viruses/g (range, 1.1 × 10^4 to 1.8 × 10^6 viruses/g; data not shown). In winter, 60 (2.2%) out of 2,723 food handlers were identified with asymptomatic NoV infection by real-time RT-PCR, 5 were identified as GI, and 55 were GII. Sequencing and phylogenetic analysis showed that 2 of 5 GI-positive samples were genotyped as GI-1 and GI-12, and 28 of 55 GII-positive samples were GII-2, GII-4, GII-6, GII-11, GII-12, and GII-14; the remaining NoV-positive samples were not genotyped (Table 1). Mean viral load in stool specimens for GI NoV was 1.87 × 10^5 viruses/g (range, 1.4 × 10^4 to 6.8 × 10^6 viruses/g) and for GII was 8.07 × 10^4 viruses/g (range, 4 × 10^2 to 4.8 × 10^5) (data not shown). Our data demonstrated that real-time RT-PCR was a more sensitive technique than conventional nested RT-PCR in epidemiological investigation. About less than 6.0 × 10^4 viruses/g was not detected by conventional RT-PCR.

The genotypic distribution of the 34 NoV strains was as follows: GII-12, 44.12% (n = 15); GII-4, 29.41% (n = 10); GII-2, 8.82% (n = 3); GII-6, 5.88% (n = 2); GII-11, 2.94% (n = 1); GII-14, 2.94% (n = 1); GI-1, 2.94% (n = 1); GI-12, 2.94% (n = 1). Molecular epidemiological studies of NoV strains circulating in asymptomatic food handlers found that GII.4, which has higher transmissibility than other genotypes, was dominant (17–19). However, in our study, the NoV GII-12 strain was more prevalent than GII-4. The infection rate of asymptomatic individuals in
nonoutbreak environments in this study and that in outbreak cases in South Korea were comparable. Approximately 18% (6/33 cases) of NoV outbreaks in South Korea are related to asymptomatic food handlers during the study period (data not shown). Among these cases, two cases occurred by the GII-12 strain. This result suggests that the population normally contains asymptomatic carriers of NoV related to the occurrence of NoV outbreaks.

Phylogenetic analysis was used to evaluate relatedness of NoV strains detected in this study and to compare them to reference strains (Fig. 1). The capsid sequence was used to describe NoV genetic diversity. Nucleotide comparison revealed that South Korean NoV GI strains showed homology of 90.2 to 97% to reference GI-1 isolates and 98.9% to GI-12 isolates. The NoV GII strains were 92.2 to 100% homologous with the reference GII-12, 88.4 to 99.2% with the reference GII-4, 96.1 to 99.6% with the reference GII-2, 90.3 to 97.3% with the reference GII-6, 87.2 to 88.4% with the reference GII-11, and 93.8 to 98.8% with the reference GII-14.

Reports of NoV surveillance in facilities that reported outbreaks are common, but studies in facilities without outbreaks are rare. The U.S. Food and Drug Administration (FDA) Food Code applies to food handlers working in food service establishments. The 2005 Food Code specifically recognized NoV as highly infectious and restricts food handling by NoV-infected asymptomatic and symptomatic employees (20).

The present study surveyed the prevalence and genotypic distribution of NoV infections in asymptomatic food handlers in South Korea, in facilities uninvolved in outbreaks. The results show that infection of asymptomatic employees as well as symptomatic food handlers may be a potential transmission source in NoV outbreaks. Given the importance of food handling in the prevention of NoV infections, food handlers should be advised to take special care to follow good kitchen hygiene practices, particularly hand washing.

**Nucleotide sequence accession numbers.** The nucleotide sequence data have been submitted to GenBank and assigned accession numbers JX623800 to JX623802.

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**REFERENCES**


