Short Communication

Combined Genogroup I and II Norovirus Infection at a Nursery

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SUMMARY: From November 2004 to April 2005, 5 cases of norovirus (NoV) occurred in Sakai City, Japan. These were all diffuse outbreaks due to infections with genogroup II genotype 4 (GII/4) virus strains. Similar outbreaks occurred throughout Japan; hence, GII/4 was assumed to be the prevalent NoV type. However, a NoV outbreak that occurred at a nursery in May 2005, was caused by infections with GI/4 and GII/6 viruses, respectively, from different children. The time course of newly infected patients showed that this nursery outbreak had a two-peak pattern, with the peak numbers of patients occurring on May 19 and May 22. Virological examination and epidemiological research could not determine whether the GI and GII NoV infections occurred at the same time, or whether there was a time difference in their appearance in the nursery. From this outbreak, it is clear that the timing of obtaining samples and obtaining the minimal necessary number of primary samples are essential for accurate epidemiological information to be obtained. In addition, we detected genotypes that were different from the previously prevalent genotypes, which raises the possibility of more frequent NoV infection or a change in the prevalent NoV genotype in this setting. In conclusion, it is difficult to predict outbreaks of NoV; however, through vigilant and early collection and analysis of later samples throughout an outbreak, it is possible to understand the prevalence and perhaps trace the source of NoV infections.

Noroviruses (NoVs) are the leading cause of acute gastroenteritis worldwide and cause outbreaks in human in various epidemiological settings, including restaurants, hospitals, cruise ships, schools, and nurseries (1). Transmission of NoV occurs through contaminated food and water, person-to-person contact, and various environmental contaminations, especially contact with dried vomit. Human NoVs are divided into two genetically distinct genogroups, genogroup I (GI) and genogroup II (GII), and the NoV strains are further subdivided into at least 15 GI and 18 GII genotypes (2). These genotypes are useful for the genetic analysis of NoV circulation worldwide or in small districts, and for determining the cause and source of infection of NoV outbreaks. From November 2004 to April 2005, 5 NoV outbreaks occurred with a GII genotype 4 (GII/4) virus strain in Sakai City, Osaka Prefecture, Japan. The situation was similar throughout Japan; hence, GII/4 was assumed to be the predominant genotype of the epidemic season of 2004-2005. However, a NoV outbreak caused by both GI and GII NoVs occurred at a nursery in May 2005.

On the 20th of May 2005, at a private nursery located in the eastern part of Sakai City, several children in a nursery class of 4-5 year-olds experienced vomiting and diarrhea. Fifteen children were absent from school due to these symptoms. On the 21st, the infection spread to other classes and the situation was defined as an outbreak of NoV (Figure 1). The nursery was composed of a total of 212 children ranging from newborns to age 5-year-olds and 38 staff members. The staff had no obvious history of eating raw shellfish. Sixty-six children (infection rate 31%) and 5 staff members (13%) were infected. A total of 10 stool samples and one vomit sample were obtained from the children and staff between May 20 and May 31. The samples were prepared as 10% emulsions with phosphate buffer and centrifuged at 11,000 rpm for 20 min at 4°C. The supernatant (140 μL) was used for RNA extraction with a QIAmp Viral RNA Mini Kit (Qiagen, Hilden, Germany), and the viral RNA was eluted in 60 μL of AVE buffer included in the kit. The NoV genome was amplified using different sets of primers; Yuri (3) and NV-SM (3) for the polymerase region, and two sets of primers, GISKF and GIISKF, and GIISKR for the capsid region (4). The results of RT-PCR testing are shown in Table 1. The genotyping and phylogenetic analysis of the products were performed as described previously (5,6).

A stool sample obtained from one child on May 20, and another 3 samples from one child and 2 staff members obtained on May 23, tested positive for GI NoV, although one staff member tested negative for GI NoV. These results showed that the outbreak in the nursery was due to a GI NoV,
but the specific bacterial pathogens could not be determined.

An additional 6 stool samples and one vomit sample were obtained from May 27 to 31: one of these samples was found to contain a GI and, unexpectedly, 3 were found to contain a GII.

Genetic analysis of the 4 GI sequences showed 95.9% homology with the GI/4 (AB042808Chiba40787JP) and all had 100% homology with each other. Similarly, the 3 GII sequences showed 93.7% homology with the GII/6 (AB039776SaitamaU397JP) and 100% homology with each other (Figure 2).

The time course of infected patients showed that there were two peaks: one on May 19 and the other on May 22 (Figure 1). There were two children with diarrhea on May 16; however, we could not determine whether these were the index cases, because stool samples were not obtained. We also could not determine whether GI and GII NoV infection occurred at the same time, or sequentially in this single nursery school.

It is clear that the collection of samples from the index case is essential in analyzing any NoV outbreak. Moreover, in determining whether an outbreak is due to one or more microorganisms, a large number of primary samples are necessary. A prompt and organized sample collection would solve these problems.

There are a few reports of combined GI/GII NoV infections in nursery outbreaks (7-9). A number of outbreaks in elementary schools and nurseries in Osaka in May were reported to be caused by various NoV genotypes, i.e., GI/3, GII/2, GII/3, GII/4, and GII/6, which are different from the previously prevalent NoV genotypes (10). This may be due to the increasing prevalence of various NoV strains with the ability to survive in environments throughout the world; such prevalence, in turn, could have resulted in the introduction of genomic changes into the NoV genome. Coexistence of multiple genotypes has been reported; however, these outbreaks were all shellfish-related infections. In such cases, it is difficult to determine whether or not there is a combined infection, because it is well known that shellfish concentrate various genotypes of NoV. There was no evidence of shellfish-related infection in the present cases.

In conclusion, it is difficult to predict outbreaks of NoV. However, through early and continued sample collection and analysis of prevalent NoV genotypes, it is possible to understand the transmission and prevalence of specific NoV genotypes and to determine if new genotypes are emerging.

### Table 1. Detection of NoV by RT-PCR from clinical samples including one vomit

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Sample</th>
<th>Sample No.</th>
<th>Date of sample collected</th>
<th>Capsid region primer GI I</th>
<th>GII</th>
<th>Primer G I</th>
<th>GII</th>
<th>Polymerase region Primer Yuri</th>
<th>NV-SM</th>
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<tr>
<td>5</td>
<td>stool</td>
<td>20050520v54</td>
<td>May 20</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>stool</td>
<td>20050520v56</td>
<td>23</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>stool</td>
<td>20050520v57</td>
<td>23</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td></td>
</tr>
<tr>
<td>30</td>
<td>stool</td>
<td>20050520v58</td>
<td>23</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>vomit</td>
<td>20050520No.10</td>
<td>27</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
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<tr>
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<td>stool</td>
<td>20050520No.11</td>
<td>27</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td></td>
<td></td>
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<tr>
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<td>27</td>
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<tr>
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<td>+</td>
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<tr>
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</table>

Four genogroup I and three genogroup II NoVs could be detected by RT-PCR both primer sets of capsid and polymerase regions.
ACKNOWLEDGMENTS

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REFERENCES