Laboratory and Epidemiology Communications

New Variant of Norovirus GII/4 Strains Prevalent in Yokohama City, October 2006 - March 2007

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Communicated by Takaji Wakita

(Accepted July 18, 2007)

Norovirus (NV) is usually prevalent in the winter season and causes acute gastroenteritis symptoms such as diarrhea or vomiting when it infects humans. In recent years, numerous outbreaks in nursing homes for the aged have been reported (1). NV is separated into two genogroups, GI and GII; GI has at least 14 and GII 17 different genotypes (2). Globally GII strains are predominant; in particular, GII/4 has been the predominant NV genotype circulating worldwide in recent years (3,4).

From October 2006 to March 2007, 87 outbreaks of gastroenteritis caused by NV were recorded in Yokohama City, Japan. Suspected transmission modes were person-to-person infection in 93% of cases (81 outbreaks) and food-borne infection through food handlers (i.e., cooks) in 7% (6). Among the 87 outbreaks, nursing homes for the aged were most often implicated as the place of the outbreak (Table 1).

Examination of stool samples collected from October 2006 to March 2007 were performed by the TaqMan based real-time PCR with separate reactions for GI (5) and GII (6) as the primary testing. No NV GI positive sample was detected. Positive GII samples by real-time PCR were subjected to reverse transcriptase (RT)-PCR using the primer set COG2F and G2SKR (7,8).

One positive NV specimen from each outbreak was subjected to further sequence analysis by direct sequencing and phylogenetically analyzed at the 5’ end of ORF2 encoding the capsid protein. As a result, of the 84 of 87 outbreaks in which the NV GII strain could be identified, there were 3 cases of GII/2 outbreak, 4 of GII/3, 76 of GII/4, and 1 of GII/5, demonstrating that the GII/4 genotype accounted for 87% of all the outbreaks derived from NV strains in Yokohama. Furthermore, all of the outbreaks occurring in nursing homes for the aged except for two undetermined cases were due to the GII/4 genotype (Table 1). At least one stool specimen of a food handler showed positive for NV GII in each outbreak of suspected food-borne infection. It is noteworthy that no oyster-related food-borne outbreak was observed as has often been seen before (9).

Compared with GII/4 strains detected in the 2004/05 and 2005/06 seasons in Yokohama City, GII/4 strains in 2006/07 season had a different cluster (Fig. 1). According to a surveillance report (10) by Foodborne Viruses in Europe Network (FBVE Network), two new GII/4 variants (GII/4 2006a and GII/4 2006b) appeared during the 2005/06 season and became predominant in the summer of 2006. The 2006/07 season GII/4 strains circulating in Yokohama belonged to the GII/4 2006b variant by comparison of the partial capsid gene using the Norovirus database, quicktyping Region C 280b (https://hypocrates.nist.gov/hbwww/Divine-Event/Quick_typingregionC280b.HTM) produced by FBVE Network. It was confirmed that the GII/4 2006b variant was indeed prevalent in Yokohama.

These results show that the GII/4 genotype has evolved and shifted between 2005/06 and 2006/07 season, and that further research like Okada’s study (4) is needed on the genetic shift of NV strains in the protruding (P) region of the capsid protein.

Table 1. Outbreaks of norovirus infection, October 2006 - March 2007, Yokohama City, Japan

<table>
<thead>
<tr>
<th>Place of infection</th>
<th>Total</th>
<th>2006</th>
<th>2007</th>
<th>Genotypes</th>
<th>GI/2</th>
<th>GI/3</th>
<th>GI/4</th>
<th>GI/5</th>
<th>U.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nursing home for the aged</td>
<td>59 (0)</td>
<td>–</td>
<td>12</td>
<td>23</td>
<td>17</td>
<td>6</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hospital</td>
<td>8 (0)</td>
<td>–</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kindergarten /Nursery school</td>
<td>7 (0)</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Welfare facility</td>
<td>5 (0)</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>Restaurant</td>
<td>5 (5)</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Primary school</td>
<td>1 (0)</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>2 (1)</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>87 (6)</td>
<td>1</td>
<td>21</td>
<td>29</td>
<td>21</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

1) The number of parenthesis shows suspected food-borne infection number.
2) U.D., undetermined.

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Fig. 1. Phylogenetic tree of NV strains circulating in Yokohama City, Japan from an outbreak of gastroenteritis from 2004 to 2007 (● y06, strains in 2006/07 season; ■ y05, strains in 2005/06 season; ▲ y04, strains in 2004/05 season). Tree was constructed with partial nucleotide sequences of the 5’ end of ORF2 by the neighbor-joining method. Genotype classification was based on the scheme of Kageyama et al. (2). The numbers in parenthesis are the GenBank accession numbers. The accession numbers of the another reference strains are as follows; Grimsby, AJ004864; Camberwell, AF145896; Lordsdale, X86557; Miami, AF414410; Leeds, AJ277608; Mexico, U22498; Hawaii, U07611; Hillingdon, AJ277607; Snow Mountain, U70059; Amsterdam, AF195848; Norwalk, M87661.

We thank the members of Health and Social Welfare Bureau, Yokohama City for collection of specimens and investigation of information in this study.

REFERENCES