

## Original Article

# An Outbreak of Food-Borne Gastroenteritis Due to Sapovirus among Junior High School Students

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**SUMMARY:** The human sapovirus (SaV) causes acute gastroenteritis mainly in infants and young children. A food-borne outbreak of gastroenteritis associated with SaV occurred among junior high school students in Yokohama, Japan, during and after a study trip. The nucleotide sequences of the partial capsid gene derived from the students exhibited 98% homology to a SaV genogroup IV strain, Hu/Angelholm/SW278/2004/SE, which was isolated from an adult with gastroenteritis in Solna, Sweden. An identical nucleotide sequence was detected from a food handler at the hotel restaurant, suggesting that the causative agent of the outbreak was transmitted from the food handler. This is the first description of a food-borne outbreak associated with the SaV genogroup IV strain in Japan.

## INTRODUCTION

Sapoviruses (SaV), formerly known as Sapporo-like viruses, are single-stranded positive sense RNA viruses in the family *Caliciviridae*.

Acute gastroenteritis due to SaV occurs mainly in infants and young children (1). Recently, outbreaks of SaV have been reported, although these viruses are rarely associated with food-borne outbreaks, much less frequently, for example, than noroviruses (2-4). A food-borne outbreak among adult staff at an elementary school was reported in Maryland, USA, and was most likely caused by food contaminated by sick employees at the catering company (5).

The SaV genome is 7.3-7.5 kb long and encodes two or three open reading frames (ORFs). On the bases of the capsid gene, SaV strains can be divided into five genogroups, GI - GV. Of these, GI, GII, GIV, and GV infect humans, while GIII infects porcine species (6). According to a genotyping study in Japan between 1998 and 2005, the GI and GII genogroups accounted for 95% of the SaV strains (7).

Herein, we describe a food-borne outbreak of gastroenteritis associated with SaV GIV strain among junior high school students during and after a study trip. The outbreak seems to have originated from a SaV-infected food handler. This is the first report of a food-borne outbreak due to SaV in Japan.

## MATERIALS AND METHODS

**Description of outbreak:** An outbreak of gastroenteritis occurred at a junior high school in Yokohama city, Japan, affecting 60 third graders, four teachers and one cameraman during and after a study trip. On 11 May 2007, the head teacher of the junior high school reported to the Kanazawa public health center that 35 third graders were absent due to vomiting, nausea, and fever at the recommendation of the school's doctor. A total of 137 people, including 123 third graders, 11

teachers, one cameraman and two attendants went on a study trip to Nara and Kyoto, 500 km from Yokohama, from 8 May to 10 May 2007. During the trip all participants stayed at a hotel in Kyoto for two nights and ate their breakfast and dinner together, but not lunch. Stool samples were obtained from 32 students, one teacher, one cameraman, four food handlers at the hotel (two males and two females), and five male food handlers at a restaurant in Kyoto (see Table 2). The 65 students and teachers showing symptoms of gastroenteritis were asked directly about their symptoms or were interviewed by telephone. Food handlers were also asked directly about their symptoms.

**Case definition:** A case of this illness was defined as exhibiting one or more symptoms, including nausea, vomiting, abdominal pain and/or diarrhea, and vomiting and/or diarrhea, in addition to gastroenteritis.

**Bacterial culture:** Stool samples from 33 patients were cultured for *Salmonella*, *Shigella*, and *Campylobacter*.

**Real-time reverse transcription (RT)-polymerase chain reaction (PCR) for noroviruses and SaV:** The stool samples obtained were examined for norovirus and SaV. Viral RNA was extracted from a 10% fecal suspension with the RNeasy Mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Real-time RT-PCR for norovirus and SaV was carried out with a Smart Cycler II (Cepheid, Sunnyvale, Calif., USA) using a QuantiTect Probe RT-PCR Kit (QIAGEN) with separate reactions for norovirus genogroup I, II, and SaV. For norovirus, the primers and probes used were those published by Jothikumar et al. (8) for genogroup I and by Kageyama et al. (9) for genogroup II. For SaV, the primers and probe used were those published by Chan et al. (10). The reaction mixtures contained 5  $\mu$ l of template RNA, each primer at a concentration of 200nM, 100nM probe JJV1P (for norovirus genogroup I) or Ring2 (for norovirus genogroup II) or CU-SV-Probe (for SaV), and QuantiTect Probe RT-PCR buffer (QIAGEN). The reaction mixture was then subjected to a one-step assay using the following amplification conditions: (i) RT for 30 min at 50°C, (ii) 15 min at 95°C to activate *Taq* polymerase, and (iii) 45 cycles of 1 s at 94°C and 30 s at 56°C. When the primary curve crossed 30 fluorescent units, the sample was judged to

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be positive. Positive and negative controls were included in each run.

**RT-PCR for SaV:** RT-PCR was performed using primers SV-F11 and SV-R1 for the capsid region (11) and modified primers Sapp36 (5'-GTTGGGGTTGGCATCAACA-3') and SV-r-c to amplify the polymerase region (12). For the nested PCR, SV-F2 and SV-R2 primers were used to amplify the capsid region (11). The PCR products were analyzed with 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining.

**Sequence analysis:** The nucleotide sequences of the PCR products were determined using the BigDye Terminator Cycle Sequencing Kit and Genetic Analyzer 310 (Applied Biosystems, Foster City, Calif., USA). Phylogenetic analyses were conducted using MEGA version 4 (13). Multiple alignments were performed with CLUSTALW (14). A dendrogram based on nucleic acid was constructed by the UPGMA method based on Kimura's two-parameter distance matrix with 1,000 bootstrap replicates.

The nucleotide sequence of the partial capsid gene for Hu/Yokohama/16/2007/JPN was submitted to the DDBJ under the accession no. AB305049. Reference SaV strains used in this study were as follows: PEC (AF182760), London/92 (U95645), Mc2 (AY237419), Mc10 (AY237420), Mex340 (AF435812), Sapporo (U65427), Manchester (X86560), Mc114 (AY237422), Houston/27 (U95644), Ehime1107 (DQ058829), SW278 (DQ125333), Hou7-1181 (AF435814), Stockholm (AF194182), Parkville (U73124), Houston/86 (U95643), Arg39 (AY289803), Cruise ship/00 (AY289804), and Hu/Osaka/19-086/2007/JP (AB327281).

## RESULTS

Of the 137 tourists who attended the study trip, 65 developed illnesses that met the case definition. The overall attack rate was 47.4%. The specific signs and symptoms are seen in Table 1. Of the 65 patients, diarrhea and fever were the dominant signs seen in 54 and 52% of the patients, respectively, whereas vomiting was seen in 38% of the patients. Nausea was the dominant symptom, seen in 71% of the patients, and abdominal pain (54%), headache (43%), malaise (25%), and chill (15%) were also seen in the patients.

The outbreak of gastroenteritis occurred at the junior high school consisting of three grades with four classes. Third graders, but not first and second graders, showed gastrointestinal symptoms, and no differences were observed between the classes (Table 2). There was no sex-related difference in the rate of patients. Of the 65 patients, 33 (51%) were females

and 32 (49%) were males. Moreover, a cameraman who was not present at the school and only joined the study trip also exhibited symptoms. By contrast, five third graders who did not participate in the study trip had no symptoms.

On 9 May at 16:00, a student who had eaten dinner at the hotel restaurant on 8 May and breakfast at the same restaurant on 9 May, but who had no direct contact with the kitchen, started to fall ill. The dates of the onset of gastroenteritis for the tourists were from 9 to 14 May, with a peak on 11 May (Fig. 1). The epidemic curve shows one peak, and has a pattern characteristic of a single-exposure, common-vehicle outbreak. The 137 tourists dined together five times, including four times in the hotel restaurant, from dinner on 8 May to breakfast on 10 May and one time in a restaurant on 10 May in Kyoto. The food served at the hotel restaurant was suspected to have been contaminated and to have been the source of the outbreak. No food handler had an illness during or before the outbreak.

Thirty-three stool samples from the patients with gastrointestinal symptoms were used for testing. Real-time RT-PCR for norovirus was performed on the stool samples; however, none was positive for norovirus genogroup I or II. Cultures of stool samples were negative for common food-borne bacterial pathogens (*Salmonella*, *Shigella*, *Campylobacter*). Interestingly, real-time RT-PCR to detect SaV demonstrated that the stool samples collected from the patients were positive (Table 2). The results confirmed that SaV was associated with the outbreak. After the results were obtained for the patients, nine stool samples from the food handlers were examined for SaV by real-time RT-PCR. As a result, one stool sample among four from the four food handlers in the hotel at Kyoto was positive. None of the stool samples from three of the

Table 1. Clinical signs and symptoms of patients with sapovirus infection

	No. of patients (%)
Patient	65
Signs	
Diarrhea	35 (54)
Fever	34 (52)
Vomiting	25 (38)
Symptoms	
Nausea	46 (71)
Abdominal pain	35 (54)
Headache	28 (43)
Malaise	16 (25)
Chill	10 (15)

Table 2. Attack rates and the results of stool samples for sapovirus

Category	No. of tourists	No. of patients	Attack rate (%)	No. of stool samples examined	No. of sapovirus positive samples by real-time RT-PCR	
Third grader of junior high school	class 1	30	14	46.7	6	5
	class 2	30	14	46.7	8	8
	class 3	31	12	38.7	8	7
	class 4	32	20	62.5	10	10
Teacher	11	4	36.4	1	1	
Cameraman	1	1	100.0	1	1	
Attendant	2	0	0.0	0	0	
Food handler in the hotel	—	—	—	4	1	
Food handler in the restaurant	—	—	—	5	0	
Total	137	65	47.4	43	33	

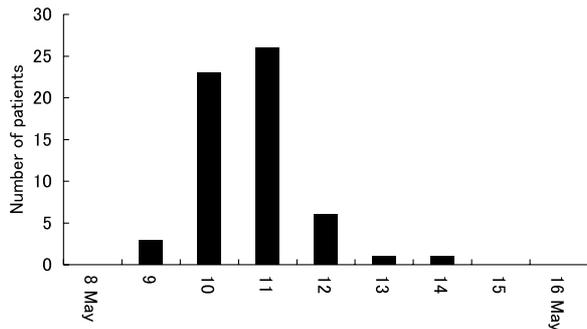


Fig. 1. Onset of gastroenteritis in 65 patients from May 9-14, 2007.

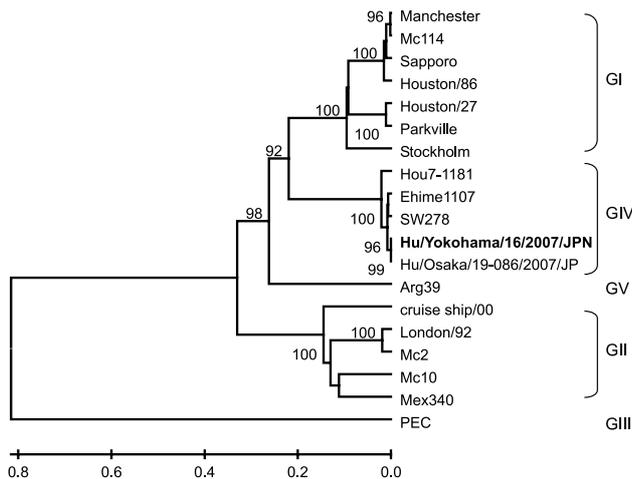


Fig. 2. Phylogenetic tree based on the partial capsid sequence. Sapovirus (SaV) strain Hu/Yokohama/16/2007/JPN detected in this study was indicated in bold letters. Reference SaV strains from GenBank were indicated in the text. The numbers in the branches indicate the bootstrap value.

food handlers in the hotel and five in the restaurant were positive for SaV.

Of 33 SaV-positive samples identified by real-time RT-PCR, six from patients ( $n = 5$ ) and a food handler in the hotel ( $n = 1$ ) were subjected to SaV-specific RT-PCR. The six SaV had identical sequences of the partial capsid region (338 bp) amplified by the primers SV-F2 and SV-R2. Six sequences were classified into genogroup IV by phylogenetic analysis when the partial capsid regions were compared (Fig. 2). The nucleotide sequences among the six strains were also identical in the partial polymerase region (638 bp), indicating that the outbreak at the junior high school was caused by a single SaV strain. The nucleotide sequence homologies between these strains and SaV GIV Hu/Angelholm/SW278/2004/SE were 98 and 96% in the partial capsid and polymerase regions, respectively. A BLAST search in the partial capsid region revealed that the strain in GenBank most closely related to the Hu/Yokohama/16/2007/JPN was from a case in Osaka, Japan (accession no. AB327281). It exhibited 100% identity at the nucleotide level.

## DISCUSSION

The human caliciviruses, norovirus and SaV, are the leading causes of acute non-bacterial gastroenteritis. A number of outbreaks of gastroenteritis associated with norovirus have occurred and been reported, whereas SaV outbreaks are rarely reported (2-5,15-17). Hirakata et al. (18) recently reported

that gastroenteritis outbreaks among tourists due to noroviruses have occurred as a result of food contamination originating from food handlers. The outbreak described here is thought to be unique in that the tourists probably developed gastroenteritis via exposure to SaV in the restaurant at the hotel where they stayed.

We described an outbreak of gastroenteritis associated with SaV at a hotel in Kyoto, Japan. In this study, symptoms associated with SaV infection were observed in 47.4% (65/137) of the subjects (Table 2). The most frequent symptoms and signs were nausea, diarrhea, abdominal pain, and fever (Table 1). In contrast to norovirus infection, where vomiting is frequently seen as the dominant sign (19), we observed diarrhea in 54% of the patients compared with vomiting in 38% of the patients, a finding which was similar to that of Johansson et al., who observed diarrhea in 72% and vomiting in 56% of SaV-infected patients (2). Moreover, fever and headache were also frequently observed among our patients. These symptoms were in accordance with calicivirus. On the other hand, the SaV gene was detected in stool samples collected from the patients by SaV-specific real-time RT-PCR. Norovirus was not detected by genogroup I- and II-specific real-time RT-PCR, and nor were any pathogenic bacteria. As no other pathogen was found, and the symptoms were consistent with SaV, it is highly likely that the outbreak was caused by SaV.

In our outbreak, a SaV-infected asymptomatic food handler was found at the hotel. Ozawa et al. (20) found that norovirus-genogroup-II-infected asymptomatic individuals had mean viral loads similar to those of symptomatic individuals, but further study is needed to clarify whether similar viral loads in asymptomatic individuals can occur in the case of SaV.

Six SaV-positive samples, including five patients and one food handler, exhibited identical nucleotide sequences in both the partial capsid and polymerase regions. Phylogenetic analysis of the partial capsid region showed a close relationship to the SaV GIV SW278 strain, which was detected in 2003 in Sweden (Fig. 2). Moreover, the nucleotide sequence in the partial polymerase region closely matched the SW278 sequence. Hansman et al. (21) identified the SW278 strain as an intergenogroup recombinant strain with genogroup II in the nonstructural region and with genogroup IV in the structural region. These results show that our SaV strain may be a recombinant strain.

Interestingly, a new SaV strain identical to our strain was submitted to GenBank (strain Sapovirus Hu/Osaka/19-086/2007/JP: accession no. AB327281) from Osaka, which is geographically close to Kyoto, Japan. This strain was isolated on 19 May, soon after our outbreak, indicating that the strain described in this study was likely to be an epidemic strain around the Kyoto and Osaka area.

Although we were not able to identify any specific food served at the hotel as the main source of the infection, we believe that the food served at the hotel restaurant was contaminated by a food handler. This conclusion is supported by the facts that (i) identical SaVs were detected from the food handler and patients; (ii) the tourists had no direct contact with the kitchen; (iii) there were no differences in the attack rates based on sex and class; (iv) the epidemic curve shows one peak and has a pattern characteristic of a single-exposure, common-vehicle outbreak.

Our results show that SaV is an important cause of food-borne infection with acute gastroenteritis in students and adults.

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