First Sapovirus Outbreak in Taiwan

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From Chinese version, pp,614-622

Abstract

Sapovirus and norovirus are important causes of gastroenteritis in humans. Norovirus is very active in the winter; it is the main cause of gastroenteritis outbreaks in the world. Sapovirus usually causes sporadic diseases during late spring and early summer. On May 9, 2007, a university in Taipei reported an outbreak of gastroenteritis to the Centers for Disease Control (CDC). Between May 4 and May 14, 55 students reported having nausea, vomiting and diarrhea. Stool samples from eight of the students were sent to CDC. No norovirus, rotavirus, or bacteria, regularly tested for gastrointestinal illness outbreaks, were found. Because the symptoms and incubation period were consistent with norovirus or sapovirus diseases, the samples were further tested for sapovirus. The results showed that this is the first outbreak of sapovirus gastroenteritis in Taiwan.

Keywords: sapovirus, gastroenteritis, gastroenteritis outbreaks, nucleic acid analysis

Background

Caliciviridae include sapovirus, norovirus, lagovirus, and vesivirus [1], of

Received: June 28, 2007; Accepted: Aug 21, 2007.

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which, Sapovirus and norovirus are major causes of human gastroenteritis. Norovirus is very active in the winter, causing large outbreaks of gastroenteritis in adults. Sapovirus usually cause sporadic disease in late spring to early summer; both children and adults may be affected. Symptoms of sapovirus gastroenteritis include diarrhea, vomiting, and fever. In 1977, the virus was identified from a home-based childcare gastrointestinal illness outbreak in Sapporo, Japan, therefore, the virus was named Sapporo virus [2]. In 2002, the International Committee on Taxonomy of Viruses (ICTV) renamed it Sapovirus [3]. Because there has not been much research on Sapovirus, and the available literatures consist of outbreaks of sporadic cases [2, 4-7], epidemiological information such as prevalence, disease rate or detection rate are available.

Sapovirus is a single-stranded RNA. There are five genotypes (GI-GV). Other genotype GIII which usually affects pigs, all genotypes cause disease in humans. Because the virus could not replicate in cells or animal models, electron microscope was used for identification. Under electron microscope, the virus resembles the star-of-David [8, 9]. In addition, sensitivity of electron microscope is low because the virus is only 41-46 nm in size. Furthermore, specimen processing for electron microscopy is complicated and time consuming. Therefore, laboratories have developed immunoassays or nucleic acid assays. However, few countries routinely test for sapovirus.

Nucleic acid assay is fast and sensitive. It is a good method f disease control and prevention when rapid identification is needed. The Center for Research and Diagnostics, Centers for Disease Control developed real-time RT-PCR for the detection of sapovirus in 2006 [10], which was used for gastrointestinal illness surveillance and this outbreak in a Taipei university. It is the first outbreak caused by sapovirus identified in Taiwan.

The Outbreak

On May 9, 2007, a university in Taipei reported an outbreak of gastroenteritis to the Centers for Disease Control (CDC). Since May 4, 2007, a number of students presented with nausea, vomiting, and diarrhea. By May 8, a total of 49 student were symptomatic. On May 8, representatives from the department of health collected stool samples from eight symptomatic students and sent the samples to the Center for Research and Diagnostics for testing. Students were monitored by the university. Between May 4 and May 14, a total of 55 students had gastrointestinal symptoms.

Laboratory diagnosis

Method of sapovirus testing may be divided into the following five steps:

1. RNA extraction: Using MagNA Pure LC DNA isolation kit III from Roche (Roche Diagnostics GmbH, Germany) to extract purified viral RNA. Add 20 μL of proteinase K to 250 μL of stool extract, mix, and extract RNA using MagNA Pure LC automated RNA purifier. Store RNA extract at -70°C.
2. Reverse transcription: Add 6.8 mL of viral RNA extract pellet to mixture, which contains 3 mg of random primer, 1.6 mM dNTP, 10U SuperScript II reverse transcriptase (Invitrogen), 40 U of RNAse (Invitrogen), 10 mM DTT and buffer. Total volume was 20 μL. Place the mixture at 25°C for 15 minutes, 50°C for 50 minutes, then 85°C for 5 minutes.
3. Real-time PCR: Primer dimer was designed based on similarity plot analysis of sapovirus gene [10]. There is a highly stable region between RNA-dependent RNA polymerase and capsid gene. The primer dimer and probe targeted the sequence between 5078 and 5181 [10]. The primer dimers were SaV124F, SaV1F, SaV5F, SaV1245R; the probes were SaV124TP and SaV5TP. Add 2.5

μL of viral cDNA to mixture, which contains 12.5 μL of QuantiTect Probe PCR Master Mix buffer (Qiagen), 400 nM of primer SaV124F, SaV1F, SaV5F, SaV1245R, and 200 nM of probe 200 nM SaV124TP and SaV5TP. Reaction procedure were incubate at 95°C for 15 minutes then subjected to 40 cycles of 95°C for 15 seconds and 62°C for 1 minute, using ABI Prism 7500 Fast (Applied Biosystems) sequence detector.

4. RT-PCR: the sapovirus primer targets region at the junction of RNA-dependent RNA polymerase and capsid genes [11]; they were SV-F11 and SV-R1. The product was 780 kps long. Using 2.5 μL of viral cDNA pellet, add mixture containing 200 nM of primer SV-F11 and SV-R1, 200 nM dNTP, 1.25 U TagDNA polymerase (Violet) and buffer. The total volum was 25 μL. After reaction at 94°C for 2 minutes, the mixture was subjected to 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 2 minutes, then 72°C for 10 minutes. Place 3 μL of the reaction product onto 2% SeaKem LE Agarose (BMA, BioWhittaker Molecular Application) made using 1 x Tris-acetate-EDTA (TAE). Product size was analyzed after eletrophoresis using 100V.
5. Sequence analysis: ABI PRISM (BigDye Terminator v3.1 Cycle Sequencing Kit) was used for sequence analysis. Base sequence was compared to NCBI genomic database for comparison, to identify the infectious agent.

Results and Discussion

The Center for Research and Diagnostics tested the eight stool samples collected during this large outbreak of diarrheal disease outbreak at the university for viral and bacterial cultures. Testing included *Shigella*, *Vibrio*, rotavirus and norovirus. After one week, no etiology was identified.

Investigation by the university showed that, between May 4 and May13, 55

students had gastrointestinal symptoms. No new cases appeared after May 14. Figure 1 is the epidemic curve, showing only one peak, indicating that this outbreak is likely to have been caused by a common source. Because there are sporadic cases after the peak of the infection, it is postulated that the infection source might have been from a common water source.

There are approximately 1,000 students at this university. Contracted restaurant provides meals for the students. Most students do eat breakfast, lunch, and dinner on campus. The investigation showed that of the 52 of the 55 ill students had history of eating off campus prior to becoming ill. Therefore, investigators hypothesized that the likelihood of meals from on campus restaurant causing illness is low. Because of this, no leftover food or environmental samples were sent to the laboratory for culture. Between May 10 and May 13, five students became ill. Of the three students who became ill on May 10, one person did not eat off campus. This student might have close contact, used the same bathroom, or drank from the same water source with other ill students. Because the laboratory did not receive any sample from these five students, no infectious agent could be identified to prove that these cases were associated with the previous wave of illnesses.

Of the 55 students, the average was 20 years; there were 26 men and 29 women. Clinical presentation included diarrhea (45, 81.8%), vomiting (22, 40.0%), abdominal pain (17, 30.9%), and fever (2, 3.6%). Symptoms lasted an average of 4.7 days (range 2-10 days). Clinical presentation of this outbreak is similar to viral gastroenteritis, but tests for norovirus and rotavirus were both negative. In addition, symptoms for this outbreak is less severe compared to norovirus outbreaks. The laboratory speculated and tested for sapovirus, which is in the same family as norovirus.

Real-time PCR for sapovirus [10] used a TaqMan MGb designed sapovirus probe which can detect four sapovirus genotypes, GI, GII, GIV and GV, simultaneously. For genotype GI, sensitivity when Ct is 38.90 may be 2.5 x 10 copies/tube. Real-time PCR was used on the eight stool samples from this outbreak. Of these, seven samples were positive for sapovirus. Ct were all less than 33 (figure 2). Because there has not been samples positive for sapovirus in Taiwan, to validate the result, these eight samples were subjected to RT-PCR for amplification using primer dimer (SV-F11 and SV-R1). Seven samples had reaction product (780 bps). Sequencing analysis of the reaction product was compared to NCBI genome database, indicating they were sapovirus GI (figure 3). The clinical presentation, length of illness and laboratory analysis all showed that this outbreak was caused by sapovirus.

For this outbreak, stool samples were not available from all ill students and employees. In addition, no water sample or other possible vehicles of transmission were tested. The route of transmission could not be identified base on the outbreak investigation performed by the health department. This is the first sapovirus outbreak identified in Taiwan. However, because detecting sapovirus from stool samples was not routinely performed for gastrointestinal outbreaks, there might have been outbreaks previously which were not identified. In 2005, Taiwan CDC began requiring the reporting of viral gastroenteritis outbreaks. During the early days of reporting, no etiology of outbreak was identified for nearly 40% of the gastroenteritis or food poisoning outbreaks, because hospitals or health department did not collect the correct samples or did not know that viral testing were available. By 2006, this had decreased to <5%, following much effort in education hospitals and health departments about outbreak sample collection. This incident showed that the etiology of gastrointestinal illness outbreaks in

Taiwan is revolving. Bacterial food poisoning is being replaced by viral infectious. This outbreak is a warning that outbreaks and number of cases of viral infectious are increasing, especially those caused by newer viruses. In addition, one must consider whether the changes in lifestyle and eating habits are contributing to the increase of gastrointestinal outbreaks. The surveys used for outbreak investigations need to include questions which will detect possible transmission routes for viral etiologies, to rapidly identify possible infection source. Because there are no epidemiological information on sapovirus in Taiwan, health agencies should strengthen research on the presence of sapovirus in the environment and the interaction between environment and human.

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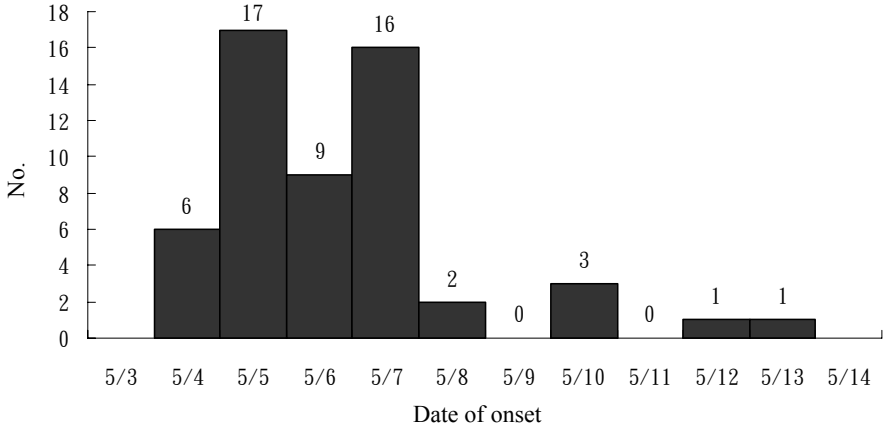

Figure 1. Epidemic curve of a gastrointestinal illness outbreak at a university in Taipei.

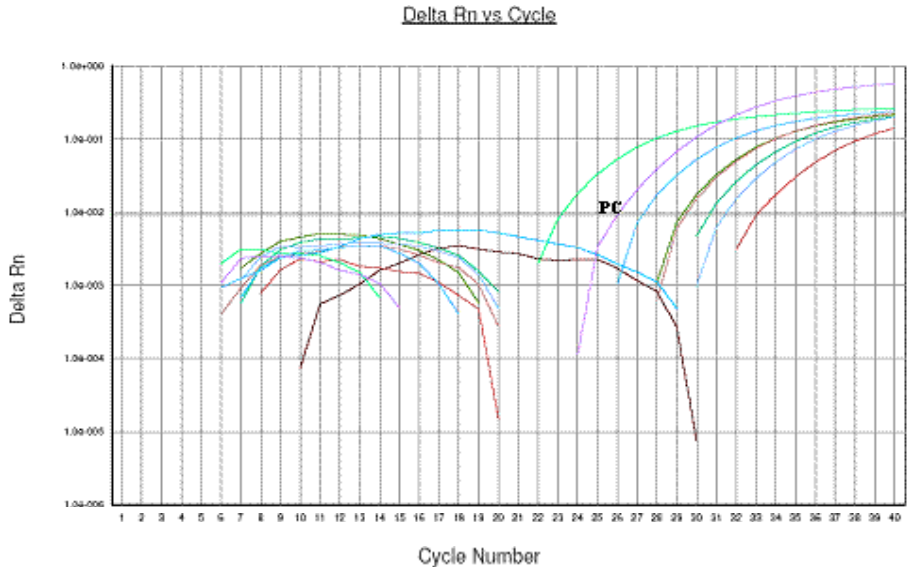

Figure 2. Result from real-time RT-PCR.

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>dbj|AB258427.1|  Sapporo virus (Hu/Chiba/041413/2004/JP) gene for polyprotein,
hypothetical protein, structural protein, partial and complete
cds
Length=2308

 Score = 1290 bits (698),  Expect = 0.0
 Identities = 698/698 (100%), Gaps = 0/698 (0%)
 Strand=Plus/Plus

Query  1    ATGGAGGGCAATGGCTCCCAGTTGCCAACCAATCAAAATGGTGGTCATGTTGGCCAGGAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ATGGAGGGCAATGGCTCCCAGTTGCCAACCAATCAAAATGGTGGTCATGTTGGCCAGGAT  60

Query  61   GTTGACCCGCCTGGCGCGACTGGTCCGACCACATCCCATGTTGTTGTGTCTAATCCAGAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GTTGACCCGCCTGGCGCGACTGGTCCGACCACATCCCATGTTGTTGTGTCTAATCCAGAA  120

Query  121  CAACCCAATGGGCCCGCACAACGCCTGGAAATGGCTGTTGCTACTGGTTCCATCCAATCA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CAACCCAATGGGCCCGCACAACGCCTGGAAATGGCTGTTGCTACTGGTTCCATCCAATCA  180

Query  181  AATGTCCCTGAAGCGATACGCAACTGCTTTGCAGTCTGTCGTACTTTTGCTTGGAATGAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  AATGTCCCTGAAGCGATACGCAACTGCTTTGCAGTCTGTCGTACTTTTGCTTGGAATGAC  240

Query  241  AGAATGCCCACTGGAACTTTCCTGGGATCTTTATCGCTTCATCCCAACATTAATCCATAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  AGAATGCCCACTGGAACTTTCCTGGGATCTTTATCGCTTCATCCCAACATTAATCCATAC  300

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Sbjct  481  ACTGATCCAGTATCATTTATGATCCCTGATGTGAGAAACATTGATTACCACAGGATGGAC  540

Query  541  ACCACTGAACCCACTTGCTCCCTTGGGTTCTGGGTCTACCAACCACTGCTAAACCCATTT  600
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Sbjct  601  TCCACCACAGCAGTCACAACATGCTGGGTGTCCATAGAAACCAAGCCTGGTGGTGATTTT  660

Query  661  GATTTTTGCCTCTTGAGGCCCCCTGGTCAACAGATGGA  698
            |||||||||||||||||||||||||||||||||||||
Sbjct  661  GATTTTTGCCTCTTGAGGCCCCCTGGTCAACAGATGGA  698
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Figure 3. Comparing sequences from positive cases to NCBI sequence database.