NOROVIRUS OUTBREAK AT A DAYCARE CENTER IN BANGKOK, 2014

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Abstract. Norovirus is a leading cause of acute non-bacterial gastroenteritis worldwide, affecting developing and developed countries, both children and adults. This study describes an outbreak of acute gastroenteritis at a daycare center of a tertiary level hospital in Bangkok, Thailand during October 2014. Although none of the staff became symptomatic, 8 of 11 children attending the center and 4 of their household contacts developed acute gastroenteritis. No pathogenic bacteria or rotavirus were detected in their evaluation; however, 3 out of 7 stool samples from the cases were positive for norovirus GII.17. Reverse transcriptase polymerase chain reaction analysis with sequence and phylogenetic analysis revealed the viral strain was the same strain reported from Taiwan in 2013. Because norovirus is a frequent cause of outbreaks in crowded conditions, early detection and preventive measures are important to control outbreaks.

Keyword: norovirus, outbreak, day-care center, GII.17, Thailand

INTRODUCTION

Noroviruses (NoV) are the leading non-bacterial cause of acute gastroenteritis (AGE) worldwide, affecting both developing and developed countries, attacking both children and adults (Yang *et al,* 2010). For children under five years of age, NoV is the second-most common cause of AGE, preceded only by rotaviruses; NoV are responsible for approximately 1.1 million hospitalizations and 200,000 deaths worldwide per year (Patel *et al,* 2008). In Thailand, NoV have been detected in hospitalized infants and children at rates ranging from 8.1% to 14.1%, and among all ages at a rate of 6.5% (Kittigul *et al,* 2010). The primary mode of transmission of the virus is the fecal-oral route, usually facilitated by close person-to-person contact, though it can also spread through contact with contaminated food, water or environmental surfaces (Kaplan *et al,* 1982; Sawyer *et al,* 1988). NoV have a low infectious dose (18-1,000 virions), are resistant to conventional cleaning agents and can remain stable on inanimate surfaces for long periods of time (Teunis *et al,* 2008). NoV outbreaks occur most commonly in crowded and unsanitary conditions, oc-

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curing in hotels, restaurants, and cruise ships, and among travelers to developing countries, military personnel and residents of healthcare facilities (Koo *et al*, 2010).

NoV belong to the *Norovirus* genus, Caliciviridae family (Xi *et al*, 1990). Similar to other viruses in this family, NoV are non-enveloped, positive-sense, single-stranded RNA viruses with a genome of approximately 7.5 kb (Xi *et al*, 1990). The genome has three open reading frames (ORFs) overlapping about twenty base pair nucleotides (Bull *et al*, 2005). ORF1 encodes six non-structural proteins: the N-terminal protein (NS1/2), NTPase (NS3), 3A-like protein (NS4), VPg viral protein genome-linked (NS5), protease (NS6), and RNA-dependent RNA polymerase (RdRp or NS7) (Green *et al*, 2007). ORF2 encodes the major capsid viral protein (VP1) which contains an N-terminal arm, a shell, or S-domain, and a protrusion, or P-domain (Prasad *et al*, 1999). The S domain (residues 1-217) is responsible for the formation of the interior shell of the capsid (Prasad *et al*, 1999). The P-domain (residues 226-530) is divided into 2 subdomains (P1 and P2), of which the P2 domain (residues 275-405) contains the most variable sequence and is located on the surface of the capsid (Prasad *et al*, 1999). Previous studies have reported the P2 domain may play an important role in immune recognition and receptor interaction, making it likely responsible for binding the capsid to histo-blood group antigens (HBGAs) (Hutson *et al*, 2002; 2003; Strong *et al*, 2012). ORF3 encodes the minor capsid protein (VP2) (Prasad *et al*, 1999; Tan *et al*, 2004).

Based on phylogenetic analysis of the viral protein capsid (VP1) sequence, NoV are classified into five genogroups (GI-GV), which are further subdivided into at least 31 genotypes (GI.1-GI.8, GII.1-GII.21, GIII.1-GIII.2) (Zheng *et al*, 2006). Three genogroups are found in humans (GI, GII and GIV) and 2 genogroups (GIII, GV) are found only in bovids and rodents, respectively (Zheng *et al*, 2006). GII.4 is the most common cause of AGE outbreaks due to NoV in humans worldwide. In Thailand, the predominant genogroups of NoV are GI and GII. The most common subtypes of GI in Thailand are GI.3 (Neesanant *et al*, 2013), GI.6 (Kittigul *et al*, 2010; Neesanant *et al*, 2013) and GI.14 (Chaimongkol *et al*, 2014). The most common subtypes of GII in Thailand are GII.3 and GII.4 (Guntapong *et al*, 2004; Chaimongkol *et al*, 2014). Other subtypes rarely detected in Thailand include GII.1, GII.2, GII.6 (Guntapong *et al*, 2004), GII.7 (Chaimongkol *et al*, 2014), GII.11 (Kittigul *et al*, 2010), GII.12, GII.13, GII.15, GII.16 (Thongprachum *et al*, 2010), GII.17 and GII.21 (Neesanant *et al*, 2013).

The purpose of this study was to investigate an outbreak of AGE that occurred at a hospital daycare center during October 2014 in Bangkok, Thailand. Based on history from the infected children and the negative findings for pathogenic bacteria and rotavirus we hypothesized the most likely cause of this outbreak was a NoV.

MATERIALS AND METHODS

This study was conducted as part of the project "Recombination Detection of Human Norovirus in Thailand during 2009-2014" and was approved by the Ethics Committee of the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (approval number IRB455/57).

On October 6, 2014, an index case was seen at our outpatient clinic with typical

symptoms of AGE: diarrhea and vomiting. She was an 11-month-old attending our hospital daycare center. Within three days, her mother also developed AGE. We suspected the possibility of an outbreak and sent a team to investigate the day-care center and discovered that three days prior to the index case developing symptoms, two other children had experienced diarrhea and vomiting. In total, our investigation found that 8 out of the 11 children who regularly attended the day-care center had symptoms of AGE along with four of their household contacts. All these cases occurred between October 1 and 17, 2014. None of the three staff members at the day-care center developed symptoms of AGE (Table 1).

To evaluate the outbreak, we defined a case of AGE as having one or more of the following symptoms: vomiting, diarrhea, abdominal cramping or nausea. Diarrhea was defined as having two or more watery or loose stools per day. To determine the causative agent, we collected stool samples from six of the eight symptomatic children and one household contact, the aunt of a symptomatic child. In total, we collected seven stool samples from patients with AGE between October 10 and 20, starting seven days after the first case. The samples were collected from either a fresh stool specimen or rectal swab and then stored at -70°C until viral testing. Bacterial pathogens were examined by routine stool culture at our hospital. Viral testing of the stool samples comprised performing reverse transcriptase-polymerase chain reaction assays (RT-PCR) to determine the presence of rotavirus and norovirus.

Rotavirus and norovirus detection

Viral RNA was extracted from 10% stool suspensions in phosphate-buffered saline (pH 7.2) using a Viral Nucleic Acid Extraction Kit (GeneAll, Songpa, South Korea), according to the manufacturer's instructions. The RNA was reverse transcribed into cDNA and screening of individual samples was accomplished using a semi-nested PCR, as described previously (Theamboonlers *et al*, 2014). Rotavirus was detected as described previously (Theamboonlers *et al*, 2014). NoV were detected using outer primers F4895 5'GAT TTA GGT GAC ACT ATA GYD STT YTC HTT YTA YGG KGA YGA TGA3' and R5591 (5'AWT CGG GCA RGA GAT YGC GAT C3') for first-round and inner primers F4895 and R5393 5'GCC TGY ACA AAR TTA TTS ATT ATC CA3' for second-round amplification.

Sequence analysis

The PCR products were gel-purified and sequenced as described previously (Theamboonlers *et al*, 2014). Nucleotide sequences were edited using the Seqman program from DNASTAR Software (version 5.0). NoV sequences found in our subjects were compared with those from GenBank using the BioEdit Sequence Alignment Editor package (version 7.0.9.0).

Phylogenetic analysis

Phylogenetic trees were produced using Clustal W alignments of partial nucleotide sequences and the neighbor-joining method with 1,000 bootstraps using MEGA (version 6).

RESULTS

All tested samples were negative for intestinal bacterial pathogens and rotavirus. Three samples were positive for NoV. The three positive samples belonged to a 7-month-old, an 11-month-old and a 30-year-old household contact (Table 1). The nucleotide sequences found in this

Table 1
Demographic data, symptoms and laboratory results of children at the day care center and their household contacts.

No.	Age	Gender	Symptoms				Specimen collection after onset of symptoms (days)	Bacteria	NoVs	Rota virus	NoV genotype	Accession no.
			Vomiting	Diarrhea	Mucus bloody diarrhea	Fever						
1	6 mo	M	Y	Y	N	Y	17	ND	Neg	Neg	-	-
2	8 mo	M	N	N	N	N	-	ND	ND	ND	-	-
3	11 mo	F	Y	Y	N	N	3	Neg	Pos	Neg	GII.17	KP726281
4[a]	30 yr	F	Y	Y	N	Y	4	ND	Pos	ND	GII.17	KP726282
5	9 mo	F	N	Y	N	N	5	Neg	Neg	Neg	-	-
6	10 mo	M	N	Y	N	Y	5	Neg	Neg	Neg	-	-
7	11 mo	M	N	N	N	N	-	ND	ND	ND	-	-
8	7 mo	M	Y	Y	N	N	1	Neg	Pos	Neg	GII.17	KP726283
9	8 mo	F	N	N	N	N	-	ND	ND	ND	-	-
10	11 mo	F	N	Y	N	N	1	ND	Neg	Neg	-	-
11	6 mo	F	N	N	N	N	-	ND	ND	ND	-	-
12	5 mo	F	N	N	N	N	-	ND	ND	ND	-	-
13[a]	3 yr	F	Y	Y	N	N	-	ND	ND	ND	-	-
14[a]	35 yr	M	Y	N	N	Y	-	ND	ND	ND	-	-
15[a]	41 yr	F	N	Y	N	Y	-	ND	ND	ND	-	-

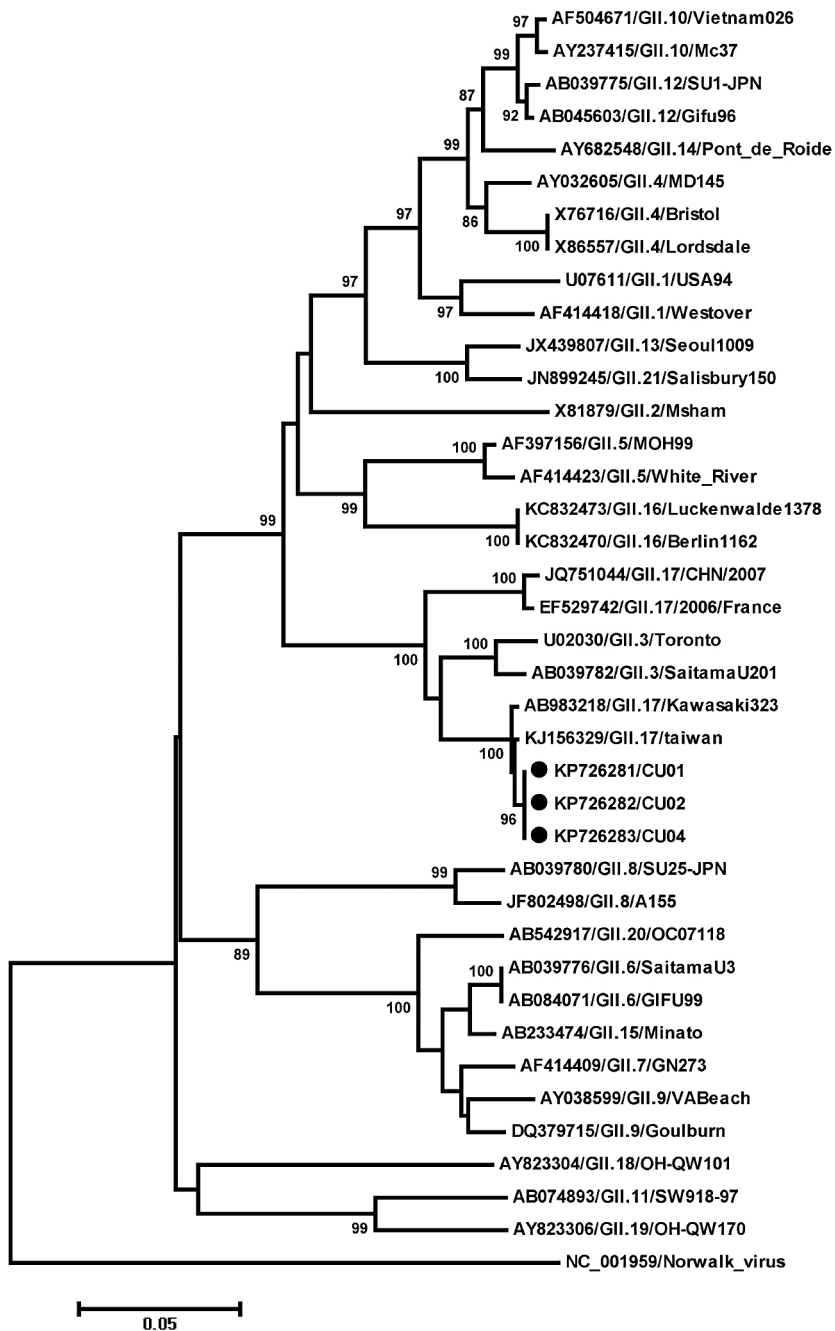[a]Contacts and household members; ND, not done.

Fig 1–Phylogenetic tree produced using Clustal W alignments of partial RdRp nucleotide sequences using the neighbor-joining method implemented in MEGA, version 6. Bootstrap resampling values are indicated at the nodes. Bootstrap values lower than 80% were omitted. Samples obtained in this study are indicated with a solid circle.

study have been stored in the GenBank database under the accession numbers KP726281-KP726283. We constructed phylogenetic trees to compare the three NoV-positive specimens with 36 representative sequences of GII genotypes from the GenBank database. Phylogenetic analysis showed the NoVs we detected shared 100% nucleotide similarity with each other and were genotype GII.17. The NoV found in our subjects were the same strain of GII.17 (KJ156329.1) as that reported from Taiwan in 2013, with the positive bootstrap percentage higher than 78% and 99% of the nucleotide sequence was identical (Fig 1). Our samples also had nucleotide similarity with sequences reported from Japan (AB983218, 98.7%), Cameroon (KJ946403, 92.8%), the Netherlands (KJ194500, 91.1) and France (EF529742, 90.5%).

DISCUSSION

This is the first time in 10 years NoV GII.17 has been documented in Thailand. This virus may have been imported from another country, such as Taiwan, but there is no way to be certain, since many factors are undocumented. The transmission of NoV GII.17 found in this study between the 11-month-old girl subject and her 30-year-old aunt proves NoV are an important contagious cause of AGE in many age groups. In this outbreak NoV may have been transmitted via contaminated environmental surfaces. Daily cleaning of the center with household detergent and frequent hand-washing with chlorhexidine soap did not prevent the outbreak from occurring. A change in disinfectant and cleaning methods used by the staff was followed by no further transmission.

A study from South Korea found NoV infections had seasonal transmission possibly due to an increase in rainfall and sewer overflow during the South Korean summer (Park *et al*, 2011). In Cameroon, NoV infections were more common during the rainy season, suggesting an association between contaminated water and NoV transmission (Ayukekbong *et al*, 2014). In our study, this NoV outbreak occurred during October, a month with the second-highest number of rainy days in Bangkok (TMD, 2014). Our study findings suggest there may be an association between an increase in contaminated water and an increase in NoV transmission.

Only three of our seven samples from symptomatic subjects were NoV-positive. This could be due to a low viral load at the end of illness caused by a delay in obtaining the samples. Samples were usually collected within four days of the onset of symptoms, which could have been enough time for the disease to pass. We did not collect samples from asymptomatic contacts. In outbreaks of AGE NoV should be considered in the differential diagnosis.

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